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Evaluation of the toxic effect of a glyphosate-based herbicide on the health and reproduction of mammals: Case of the female rat (*Rattus norvegicus)* of the W*istar* strain

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Abstract

Kalach 360 SL, a glyphosate-based herbicide used in agriculture in Côte d'Ivoire, is effective against weeds and improves crop yields. According to the UPL group, Kalach 360 SL is classified as low-hazard. However, evidence of the toxicity of the active ingredient (glyphosate) has been provided by some authors. This study aimed to evaluate the toxicity of Kalach 360 SL on mammalian health. Thirty-two rats, divided into four groups of eight, were used. The control group received distilled water. The other three groups received 0.1 mL, 0.2 mL, and 0.3 mL of Kalach 360 SL orally, corresponding to 250, 500, and 1000 mg/kg bw of glyphosate per day for 60 days. The results revealed a significant increase in relative liver mass. The relative mass of the kidney decreased significantly. The mass of the ovary decreased, unlike the mass of the uterine horn, which increased. The biochemical study revealed a significant increase in the serum concentration of ALAT, ASALT, Cholesterol, Creatinine and Urea. A decrease in the serum concentration of Total Protein was observed. Hormonal parameters revealed a decrease in the concentration of FSH and Progesterone. An increase in the concentration of Estradiol was observed. The histological study of the liver and kidney showed structural alterations within these organs. The histological study of the ovary and uterine horn revealed structural and functional alterations of these organs. These alterations are characterized by tissue lesions and necrosis. The daily use of this herbicide would present dangers to health and reproductive function.

Keywords: Kalach 360 SL; Toxicity; Biochemical Parameters; Hormonal Parameters; Liver; Ovary

1. Introduction

In recent years, labor for plantation maintenance has become very scarce in African countries. To fill this gap and improve yields, producers are most often turning to herbicides (Baldi et al., 2013) [1]. Herbicides are commonly used pesticides worldwide (Periquet et al., 2004) [2]. They belong to the large family of biocides (Periquet et al., 2004) [2]. Herbicides are used for crop protection, against herbaceous or woody plants. The associated agricultural practice is chemical weeding (Periquet et al., 2004) [2]. Depending on their mode of action, herbicides are classified into selective weedkillers, brush clearers, total weedkillers, products that destroy the aerial part of plants, anti-germs and Silicides more specifically target forest species or the natural regeneration process (Yahia, 2004) [3]. Among these different herbicides, total weedkillers are the most used in the agricultural sector (Yahia, 2004) [3]. However, the use of herbicides is problematic, because they do not only act in the desired location, but also disrupt the natural ecological balance (Camard, 2010) [4]. Indeed, the use of herbicides in agriculture can lead to the contamination of ecosystems and an impact on mammals (JardinSuisse, 2021) [5]. Kalach 360 SL, a Glyphosate-based herbicide distributed by Callivoire. It is used in Côte d'Ivoire in the agricultural sector. Kalach 360 SL is effective against weeds and improves

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crop yields. Kalach 360 SL has an LD50 greater than 2000 mg/kg body weight. Therefore, Arysta LifeScience and UPL classify it as a low-hazard product. Although less hazardous, this substance may develop some adverse effects. The objective of this study is to evaluate the toxicity of the herbicide Kalach 360 SL (Glyphosate 360 mg/l) on mammalian health and reproduction.

2. Materials

2.1. Animal Materials

The animal material consists of 32 adult male rats weighing between 120 g and 130 g. These animals all came from the ENS vivarium in Abidjan. They were fed corn, bread, and a soy + fish + corn-based formula. They were raised in polyethylene cages lined with wire-mesh wood shavings, with free access to water and food. The cages were cleaned every two days.

2.2. Chemical Equipment

The chemical used was Kalach 360 SL (Glyphosate 360 mg/L). This herbicide is distributed in Côte d'Ivoire by Callivoire. Callivoire is a subsidiary of Unité Phosphorus Limited, a global player in the plant protection industry. In Côte d'Ivoire, Kalach 360 SL is used on a wide range of crops (tomatoes, carrots, cabbage, cucumbers, rice, oil palm, cotton). It is one of the most popular herbicides among Ivorian farmers due to its effectiveness against weeds and its non-toxicity on crops.

3. Methods

3.1. Preparation of the test solution

According to Aymeric (2016) [6], the LD50 of glyphosate is greater than 8000 mg/kg of body weight. Three doses of glyphosate (Do) were defined according to the LD50: LD50/32, LD50/16, and LD50/8, i.e., 250 mg/kg, 500 mg/kg, and 1000 mg/kg of body weight.

3.2. Rat Treatment

Four groups of 8 rats each were formed. The rats were treated daily by gavage for 60 days. For each group, the animals received 1 ml of the corresponding preparation individually and daily for the group for 60 days. Thus,

- the control group received distilled water
- group 1 received 250 mg/kg of body weight of glyphosate, i.e., 0.1 ml of Kalach 360 SL + 0.9 ml of distilled water;
- Group 2 received 500 mg/kg body weight of Glyphosate, i.e. 0.2 ml of Kalach 360 SL + 0.8 ml of distilled water;
- Group 3 received 1000 mg/kg body weight of Glyphosate, i.e. 0.3 ml of Kalach 360 SL + 0.7 ml of distilled water).

3.3. Sample Collection

At the end of the test, the female rats were sacrificed after anesthesia. Blood was collected from the animals at the time of sacrifice. Blood was collected using tubes containing the anticoagulant EDTA (ethylenediamine tetraacetic acid) and dry tubes (without anticoagulant). The collected blood was used to measure biochemical parameters.

Vital organs such as the liver and kidneys were removed after opening the abdominal cavity by dissection. They were rinsed with 0.9% sodium chloride (NaCl) and then weighed. After weighing, the organs were preserved in 10% formalin. The liver and kidney were used for histological study, which will reveal the effects of Kalach 360 SL on the internal structure of the kidney and liver.

Reproductive organs such as the ovary and uterine horn were removed by dissection. They were rinsed with 0.9% sodium chloride (NaCl) and weighed. After weighing, the organs were preserved in 10% formalin. The testicle was used for the histological study.

3.4. Determination of Biochemical Parameters

Biochemical assays were performed using a Mindray BS-200E automatic analyzer from China. The assay methods differ depending on the biochemical parameters being measured. The assay device is configured for each biochemical

parameter. Thus, AST, ALT, total protein, bilirubin, triglycerides, total cholesterol, urea nitrogen, and creatine are measured.

3.5. Determination of Hormonal Parameters

The principle of assaying gonadal hormones (testosterone) combines the competitive method with final fluorescence detection (ELFA). To do this, the 60-test kit (VIDAS, BIOMERIEUX, France) containing all the immunological reaction reagents are used. The testosterone assay was performed using the HITACHI 902, Automatic analyzer, Japan. The sample to be tested is collected and then transferred to the well containing the testosterone derivative. A competition is carried out between the hormone present in the serum and the estradiol derivative of the conjugate. At the end of the test, the results are calculated by the machine compared to a stored calibration curve, then printed.

The principle of assaying pituitary hormones (LH and FSH) combines the sandwich enzyme immunoassay method with a final fluorescence detection (ELFA). The assay steps are the same as for gonadal hormones except that the antigen (hormone) binds on the one hand to the immunoglobulins fixed on the cone and on the other hand to the conjugate, thus forming a "sandwich".

3.6. Histopathology of the Liver, Ovary, and Uterine Horn

The liver, ovary, and uterine horn were removed and preserved in fixative (10% formalin + 9% NaCl) for 48 hours. Once removed from the fixative, these organs were rinsed and placed in perforated cassettes. The cassettes were labeled and successively placed in three alcohol baths of increasing strength (80° in 1 hour, 90° in 2 hours, and two baths of 100° in two hours each). After dehydration, the organs were placed in three successive toluene baths. The first bath lasted 1 hour, and each of the other baths lasted two hours. After passing through the toluene baths, the samples were directly immersed successively in two baths of molten paraffin. This process was carried out in an incubator (Memmert, Germany) between 58 and 60°C. The first bath lasts two (02) hours and the next one is done in three (03) hours. After impregnation, the cassettes are opened, the organs are removed and then placed in molds containing a thin quantity of paraffin. The mold was covered by the cassette inside which the liquid paraffin is poured until filling. Cooling, solidification of the molds and demolding reveals paraffin blocks in which the organ fixed to the back of the cassette is embedded. The formed blocks are roughened using the Leica RM2125 RTS brand microtome (Germany) and placed on ice for 30 min. After this step, the blocks are mounted on the microtome and then undergo 4 to 5 µm thick sections.

3.7. Statistical Data Analysis

Graph Pad Prism7 software was used for statistical data analysis. Results are presented as mean plus or minus standard error (MSE). Data were separated using the one-way ANOVA method, followed by Tukey's multiple comparison test at the 5% significance level. Differences are considered:

- Not significant if $p > 0.05$
- * Significant if $p < 0.05$

4. Results

4.1. Effect of Kalach 360 SL on the relative masses of vital and reproductive organs

The relative liver masses of female rats treated with doses of 250, 500, and 1000 mg/kg body weight of glyphosate were 2.677 ± 0.060; 3.050 ± 0.092; 3.444 ± 0.130 g/100g versus 2.677 ± 0.060; 3.050 ± 0.092; 3.444 ± 0.130 g/100g for control female rats. The results reveal a significant increase ($p < 0.05$) in liver mass for female rats treated with the dose of 1000 mg/kg body mass of Glyphosate compared to control female rats (Table I).

In contrast, in the kidneys, the relative mass is respectively 0.503 ± 0.023; 0.507 ± 0.020; 0.508 ± 0.025 g/100 g for the groups treated with doses of 250, 500 and 1000 mg/kg body weight of Glyphosate and 0.684 ± 0.058 g/100 g for the control group. The results show a significant decrease ($p < 0.05$) in female rats of the treated groups compared to the control rats (Table I).

The values of the relative masses of the ovary of female rats treated with doses of 250, 500 and 1000 mg/kg body weight of Glyphosate are 0.035 ± 0.003; 0.035 ± 0.005 and 0.025 ± 0.003 g/100g body weight versus 0.051 ± 0.003 g/100g body weight for control female rats. The results showed a significant decrease ($p < 0.01$) in the relative mass of the ovaries in female rats treated with 1000 mg/kg bw doses of Glyphosate compared to the control (Table I).

For doses of 250, 500 and 1000 mg/kg bw of Glyphosate, the relative masses of the uterine horns were 0.314 ± 0.097; 0.312 ± 0.012; 0.406 ± 0.043 g/100 g body weight versus 0.176 ± 0.012 g/100 g body weight for the control group. Statistical analysis showed a significant increase in the relative mass of the uterine horns in all treated groups compared to the control group (Table I).

Table 1 Relative mass of organs in female rats, control and treated with Kalach 360 SL at doses of 250, 500, 1000 mg/kg body weight of Glyphosate

Glyphosate concentration (mg/kg m.c)	**Relative mass of organs (g/100g of body weight)**			
	Liver	Kidney	Ovary	Uterine horn
0	2.677 ± 0.060	0.683 ± 0.058	0.051 ± 0.003	0.176 ± 0.012
250	2.677 ± 0.060	0.503 ± 0.023*	0.035 ± 0.003	0.314 ± 0.097*
500	3.050 ± 0.092	0.506 ± 0.020*	0.035 ± 0.005	0.312 ± 0.012*
1000	3.444 ± 0.130*	0.507 ± 0.025*	0.025 ± 0.003*	0.406 ± 0.043*

Not significant if $p > 0.05$, Significant if $p < 0.05$ *, Vertical reading direction

4.2. Effects of Kalach 360 SL on Biochemical Parameters

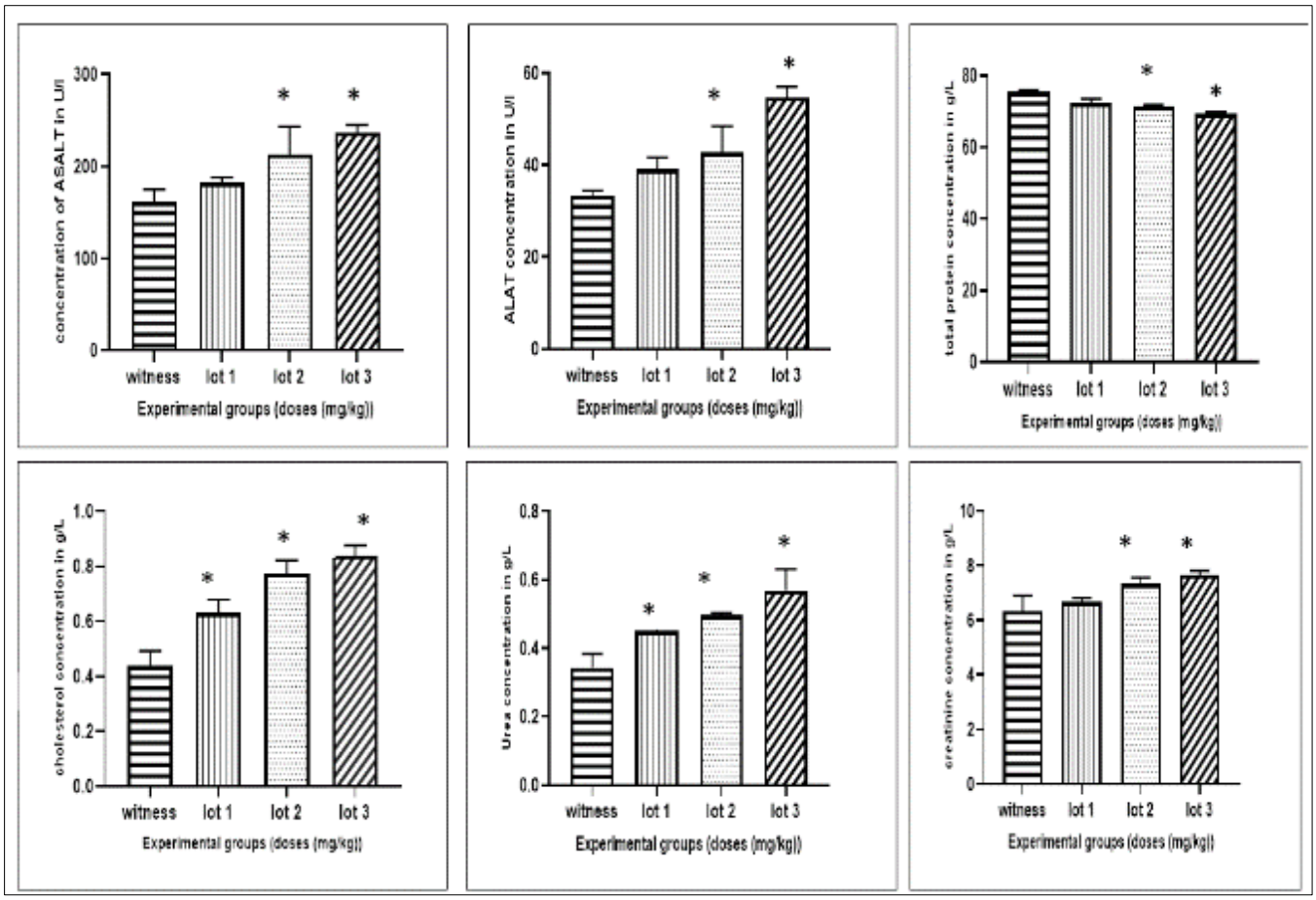
Liver parameters such as ALT, AST, and Total Protein were measured. The results of the data analysis from this assay are shown in Figures 1 and 2. Serum AST concentrations in the groups treated with doses of 250, 500, and 1000 mg/kg body weight were 182.300 ± 3.180 U/L; 213.000 ± 17.500 U/L; 236.700 ± 4.702 U/L; and 161.300 ± 7.667 U/L for the control group. The results showed a significant increase ($p<0.05$) in serum AST concentrations in rats treated with 500 and 1000 mg/kg doses compared to control female rats (Figure 1).

For doses of 250, 500 and 1000 mg/kg body weight, serum ALT concentrations in treated female rats were 39.000 ± 1.528 U/L; 42.670 ± 3.333 U/L and 54.670 ± 1.333 U/L, respectively, compared to 33.330 ± 0.667 U/L for control female rats. Statistical analysis showed a significant increase ($p<0.05$) in serum ALAT concentration in female rats treated with doses of 500 and 1000 mg/kg body weight compared to control female rats (Figure 1).

The serum Total Protein concentration in the groups treated with doses of 250, 500 and 1000 mg/kg body weight are 72.330 ± 0.667 U/l; 71.330 ± 0.333 U/l; 69.330 ± 0.333 U/l and 75.330 ± 0.333 U/l for the control group. The results showed a significant increase ($p < 0.05$) in the serum concentration of Total Protein in female rats treated with doses of 500 and 1000 mg/kg compared to control female rats (Figure 1).

The serum concentration of Cholesterol in the groups treated with doses of 250, 500 and 1000 mg/kg of body mass are 0.630 ± 0.030 U/l; 0.773 ± 0.028 U/l; 0.400 ± 0.021 U/l compared to 0.436 ± 0.032 U/l for the control group. The results showed a significant increase in the serum concentration of Cholesterol in female rats treated with doses of 250, 500 and 1000 mg/kg compared to control female rats (Figure 1).

For doses of 250, 500 and 1000 mg/kg body weight, serum Urea concentrations were respectively 0.450 ± 0.010 U/l; 0.496 ± 0.003 U/l; 0.570 ± 0.035 U/l versus 0.343 ± 0.023 U/l for the control group. The results also show a very significant increase ($p<0.05$) in serum urea concentration in female rats treated with the dose of 250, 500 and 1000 mg/kg body weight compared to the controls (Figure 1). The serum creatinine concentration in the groups treated with doses of 250, 500 and 1000 mg/kg body weight were 6.667 ± 0.333 U/l; 7.333 ± 0.333 U/l; 7.667 ± 0.667 U/l and 6.33 ± 0.667 U/l for the control group. The results showed a significant increase in the serum creatinine concentration of female rats treated with doses of 500 and 1000 mg/kg compared to the control female rats (Figure 1)



Not significant if p > 0.05, Significant if p < 0.05.

Figure 1 Variation in serum concentration of biological markers in control and Kalach 360 SL-treated female rats at doses of 250, 500, 1000 mg/kg b.w. of Glyphosate

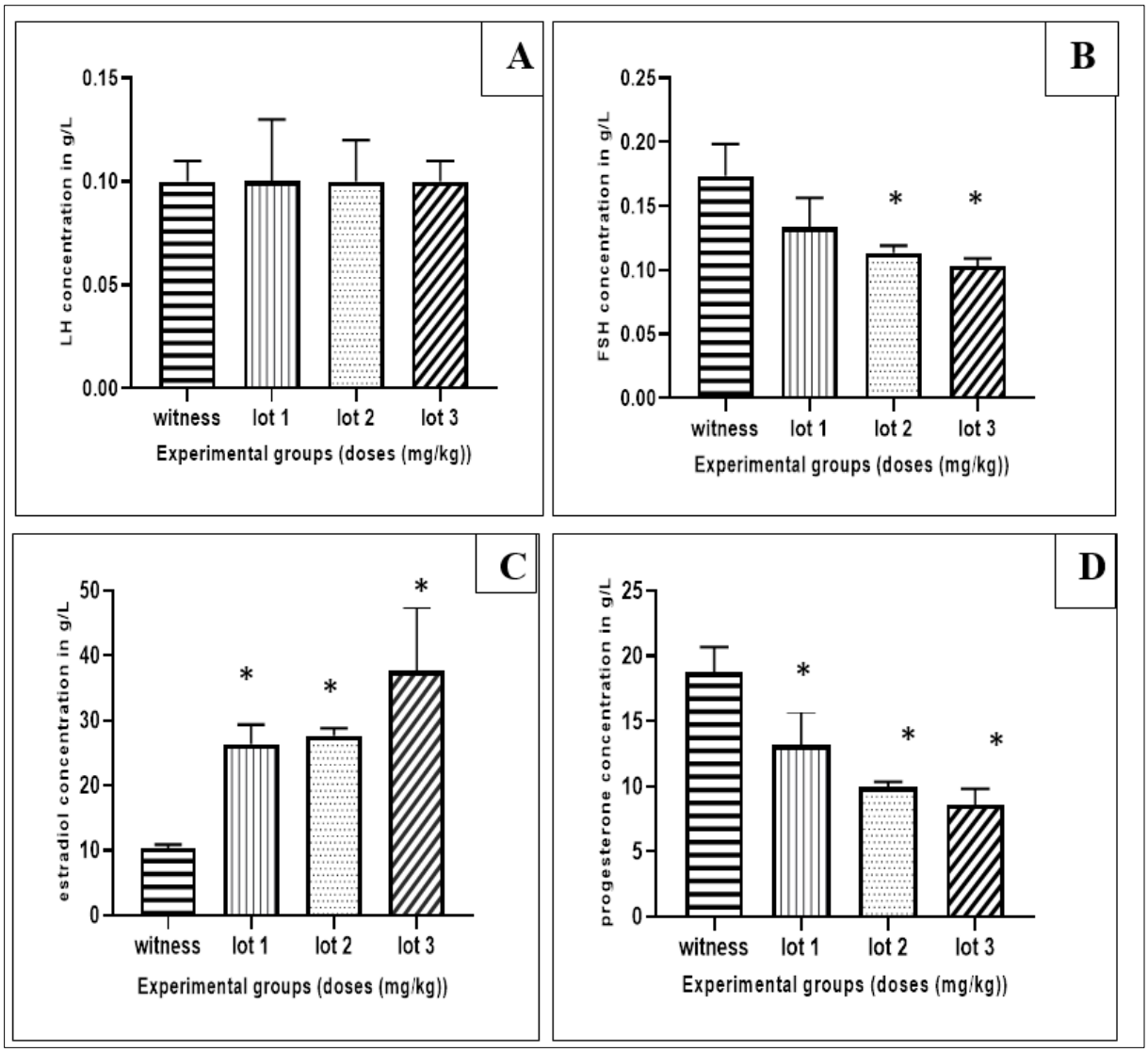
4.3. Effects of Kalach 360 SL on Hormonal Parameters

The results revealed no significant changes ($p>0.05$) in serum LH concentrations in all treated groups compared to the control group (Figure 2).

Groups treated with 500 and 1000 mg/kg bw doses of Glyphosate had low FSH levels compared to the control group. Statistical analysis showed a significant decrease ($p<0.05$) in serum FSH concentrations in female rats treated with 500 and 1000 mg/kg bw doses of Glyphosate compared to the control group of female rats (Figure 2).

Groups treated with the respective doses of 250, 500, and 1000 mg/kg bw of Glyphosate all had elevated estradiol levels. Statistical analysis revealed a significant increase ($p<0.05$) in serum estradiol concentrations in the treated groups compared to the control group (Figure 2).

Groups treated with the doses of 250, 500, and 1000 mg/kg bw of Glyphosate had low progesterone levels. The results showed a significant decrease ($p<0.05$) in serum progesterone concentrations in all groups of female rats treated with Kalach 360 SL compared to the control group (Figure 2).

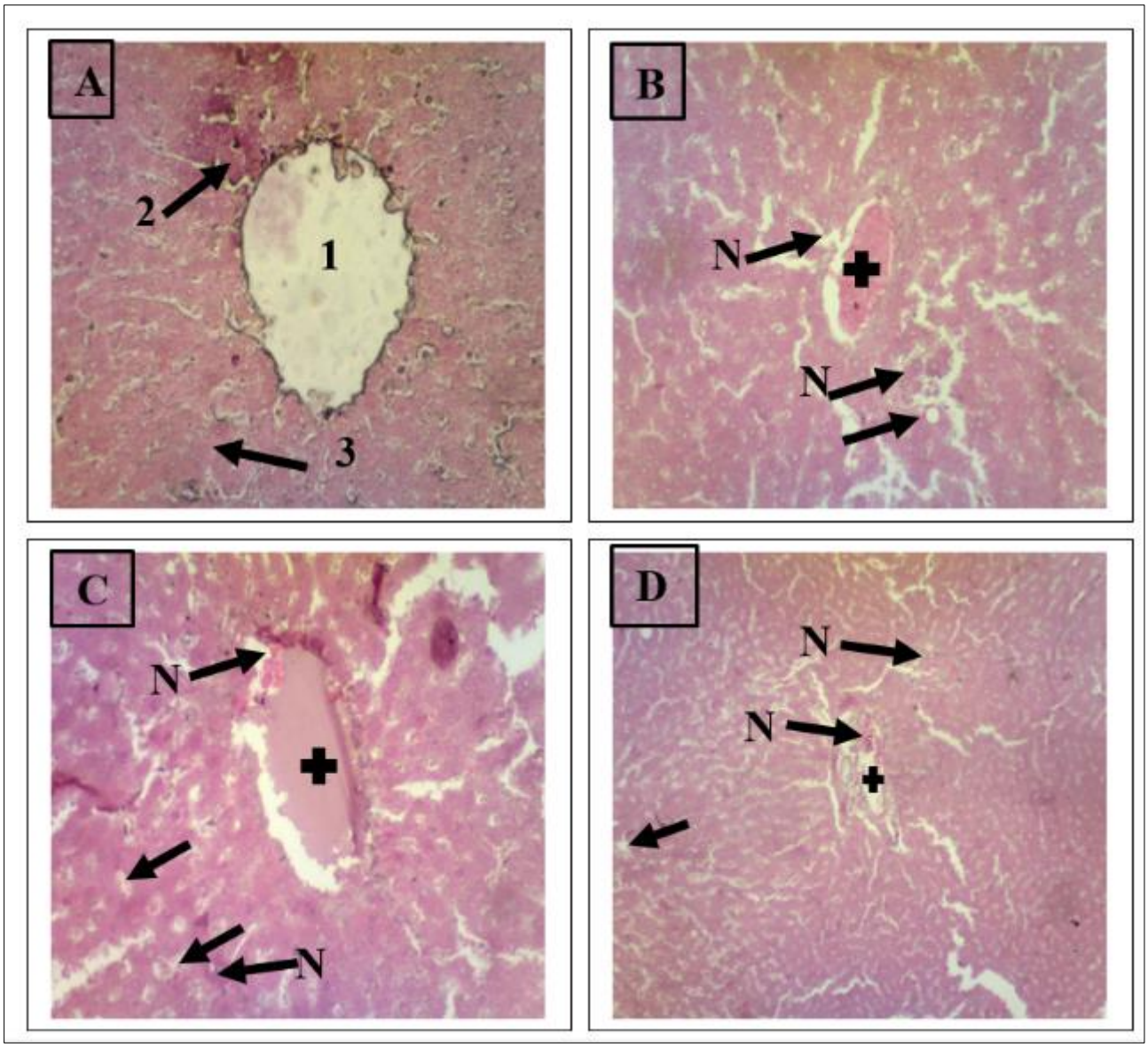


A: Proportion of LH, **B**: Proportion of FSH, **C**: Proportion of Estradiol, **D:** Proportion of Progesterone; Not significant if $p > 0.05$, * Significant if $p < 0.05$

Figure 2 Proportions of Dosed Hormones

4.3. Impact of Kalach 360 SL on Liver Histology

Microscopic observation of histological sections in control female rats shows normal architecture. Liver cells (hepatocytes) are arranged and arranged around the centrilobular vein (Figure 3 A). In treated female rats, the presence of hepatic necrosis, inflammatory cell infiltration, hepatocyte vacuolation, and congestion of the centrilobular veins is observed (Figures 3 B; 3 C; 3 D). These observations are absent in control female rats.

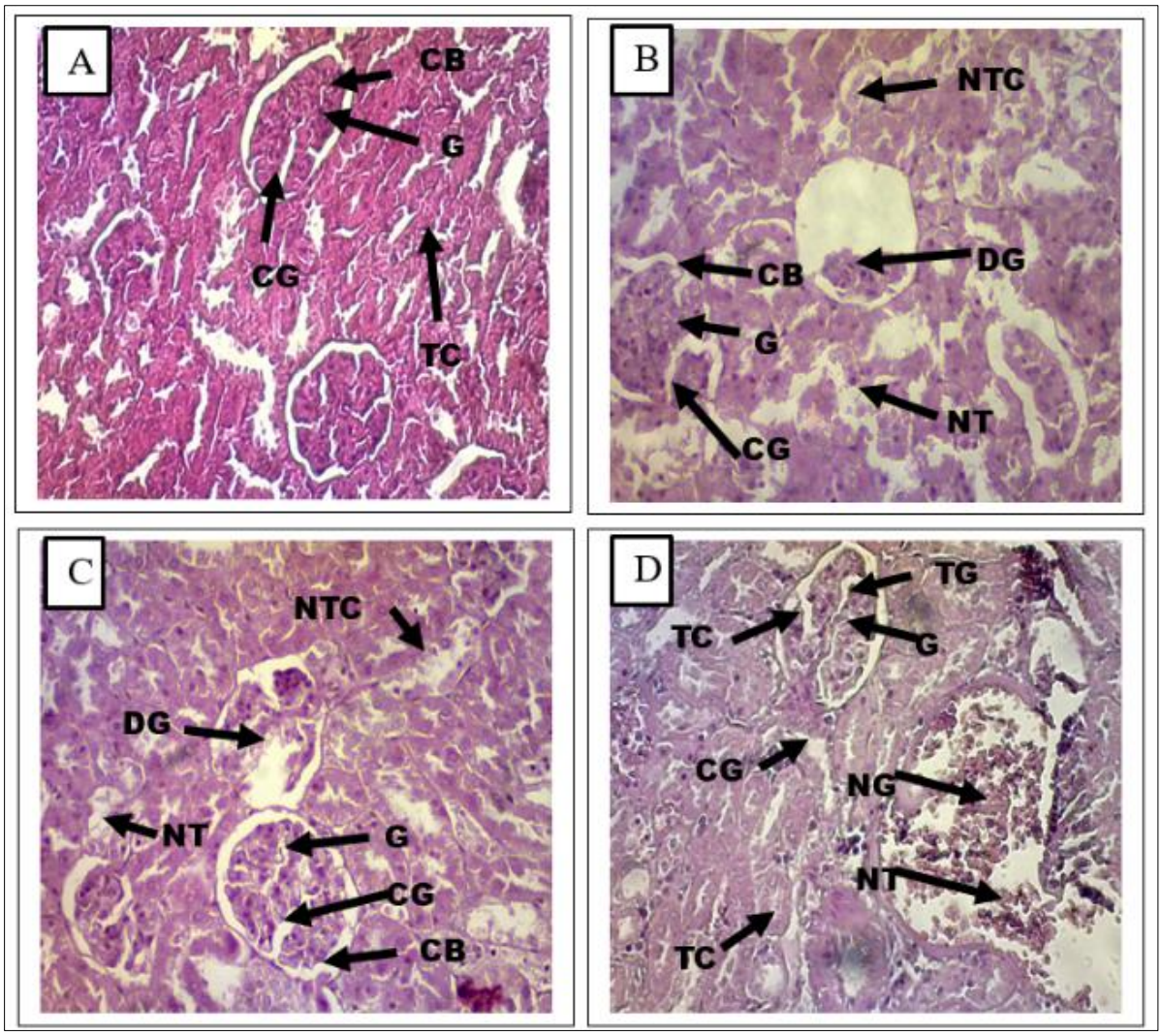


A: control; **B:** treated with 250 mg/kg bw; **C:** treated with 500 mg/kg bw; **D:** treated with 1000 mg/kg bw.1: Centrolobular vein; 2: sinusoidal capillaries; 3: hepatocytes; Vascular congestion; Hepatocyte vacuolation; **N**: Cell necrosis

Figure 3 Histological sections of the liver of female rats treated with Kalach 360 SL (A) and control rats (B, C, D)

4.4. Effects of Kalach 360 SL on Kidney Histology

Histology reveals normal kidney architecture in control female rats (Figure 4 A). Indeed, the observation shows no inflammation, necrosis, or tissue lesions. In female rats from the treated group, lesions within the tissue and necrosis in the glomerulus are observed (Figures 4 B; 4 C; 4 D).

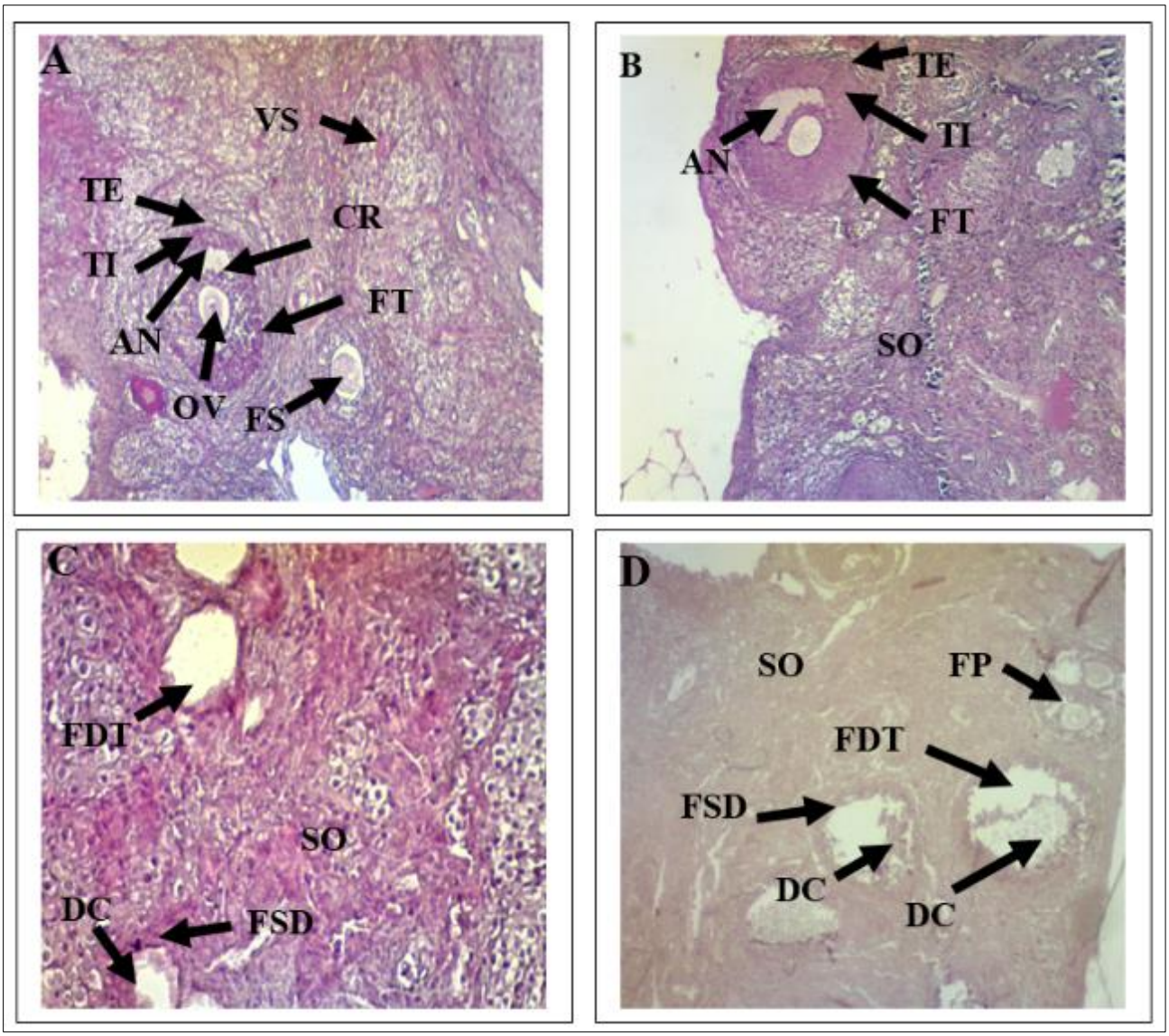


G: Glomerulus; CB: Bowman's capsule; CG: Glomerular duct; TC: Convoluted tubule; NTG: Glomerular tissue necrosis; NT: Tissue necrosis; NCG: Glomerular duct necrosis; NTC: Convoluted tubule necrosis; R: Convoluted tubule diameter narrowing; NTG: Glomerular tissue necrosis; DG: Lomerulus destruction

Figure 4 Histological sections of the kidney of control female rats (A) and female rats treated with Kalach 360 SL solution (B, C, D) at respective doses of 250, 500, 1000 mg/kg b.w. of Glyphosate

4.5. Impact of Kalach 360 on Ovarian Histology

The histology of the ovaries in the control group (Figure 5 A) exhibits normal structural architecture. The presence of primary, secondary, and tertiary follicles, as well as the corpus luteum, is observed. These follicles develop correctly, with a high presence of follicular and egg cells. There is an absence of lesions and necrosis in the ovarian and follicular tissue. However, in the treated female rats (Figure 5 B, C, D), the ovarian structure exhibits abnormalities. The absence of eggs and corpus luteum, as well as a decrease in primary follicles, is noted. Ovarian tissue lesions and destruction of secondary and tertiary follicles were recorded. Follicular cell necrosis followed by cellular debris deposits were also observed.

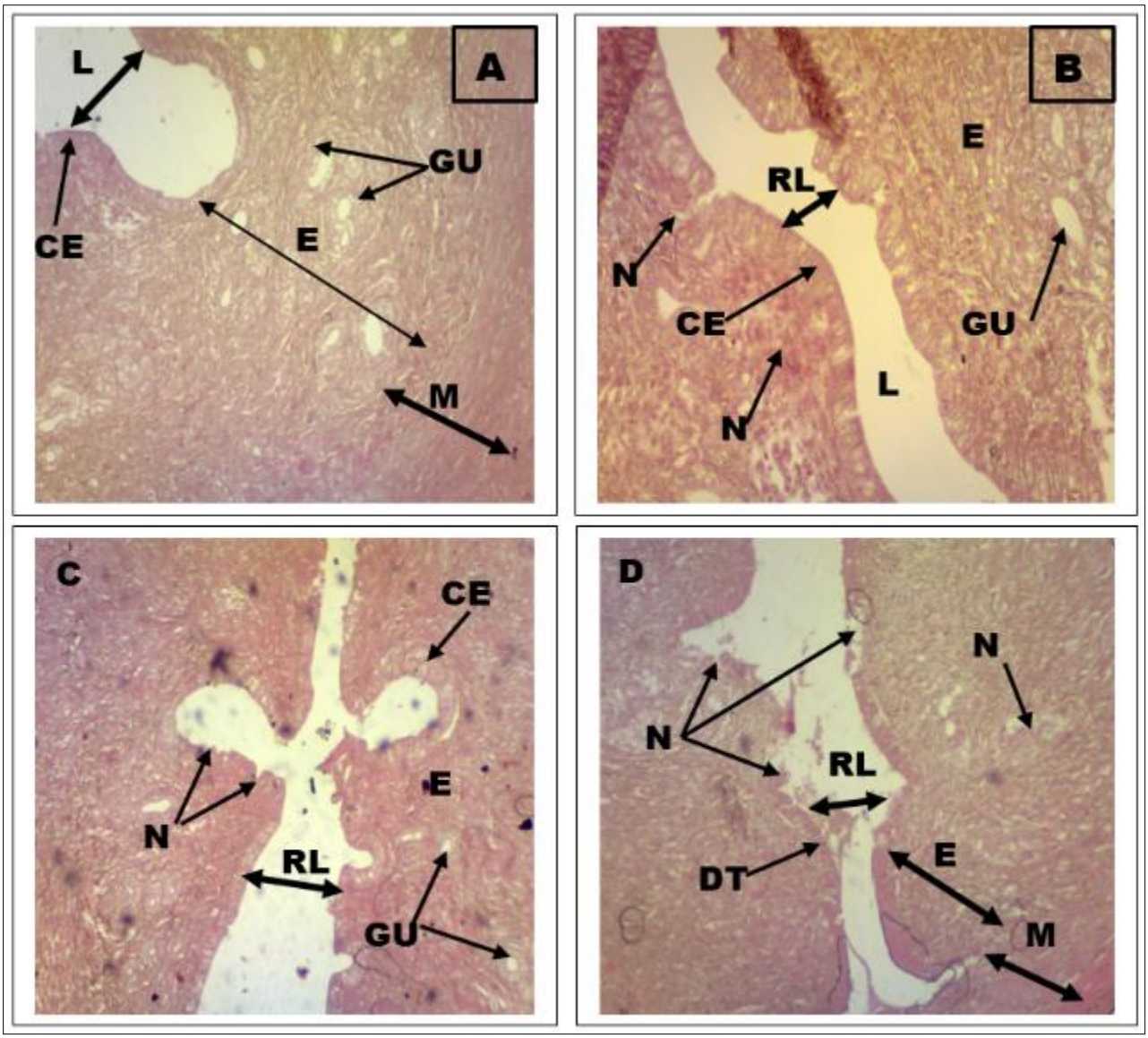


A: control; B: treated (250 mg/kg bw), C: treated (500 mg/kg bw), D: treated (1000 mg/kg bw); TI: theca interna; TE: theca externa; AN antrum; O: ovum; CJ: corpus luteum; CR: corona radiata; FT: tertiary follicle; FS: secondary follicle; FP: primary follicle; FTD: destroyed tertiary follicle; FSD: destroyed secondary follicle; DC: cellular debris; VS: blood vessel; SO: ovarian stroma

Figure 5 Histological sections of the ovary of control (A) and treated (B, C, D) female rats with Kalach 360 SL

4.6. Effects of Kalach 360 SL on the Histology of the Uterine Horns

Histological studies performed on the uterine horn of control female rats (Figure 6 A) reveal normal architecture. Indeed, we observe normal endometrial tissue and thickness. The myometrium also exhibits normal structure and thickness. A strong presence of uterine glands and epithelial cells is observed. In contrast, in treated female rats (Figure 6 B, C, D), some disorganization of the uterine tissue structure is observed. These abnormalities are characterized by a reduction in the lumen and thickness of the endometrium and myometrium. Necrosis is observed in epithelial cells and endometrial tissue. A significant reduction in uterine glands and the presence of tissue debris is observed.



A: control; **B**: treated (250 mg/kg bw); **C:** treated (500 mg/kg bw); **D:** treated (1000 mg/kg bw); L: lumen; EC: epithelial cells; E: endometrium; M: myometrium; GU: uterine gland; RL: narrowing of the uterine cavity lumen; N: necrosis; DT: tissue debris

Figure 6 Histological sections of the uterine horn of control (A) and treated (B, C, D) female rats with Kalach 360 SL

5. Discussion

5.1. Effect of Kalach 360 SL on the relative masses of vital and reproductive organs

The absolute and relative mass of these organs is one of the major indicators for assessing toxicity. Oral administration of Kalach 360 SL to female rats over 60 days resulted in significant changes in organ mass. In the liver, this increase was significant for male rats treated with doses of 500 and 1000 mg/kg and for female rats treated with a dose of 1000 mg/kg of body weight of glyphosate. Treatment of female rats with Kalach 360 SL reportedly resulted in liver hypertrophy. This liver hypertrophy is thought to be caused by the accumulation of these products in the liver as well as the liver's role in xenobiotic metabolism (Yahia, 2016) [3]. According to Kadota et *al*. (1976) [7], this increase in liver mass could be caused by the increased demands for detoxification of toxic compounds (Kadota et *al.* 1976) [7]. A study revealed an increase in liver weight after treatment with doses of 1.30 and 13 mg/kg of Metribuzin (herbicide) for 14 weeks in female and male rats (Chiali et *al*., 2013) [8]. Our results also revealed a significant decrease in kidney mass in female rats treated with doses of 250, 500 and 1000 mg/kg body weight of Glyphosate. The administration of Kalach 360 SL would impact the relative mass of the kidney and kidney function.

The results showed a significant change in the mass of the reproductive organs of female and male rats. Indeed, a decrease in ovarian mass was observed in female rats treated with Kalach 360 SL. Exposure of female rats to Kalach 360 SL (360 g/L of glyphosate) is reported to cause gonadal atrophy. The ovaries are the organs in which eggs are produced. In addition to their egg-producing functions, the ovaries are part of the endocrine system through the synthesis of sex hormones (Sisodia and del Carmen, 2022) [9]. Kalach 360 SL is reported to disrupt reproduction in female rats by reducing ovarian mass.

5.2. Effects of Kalach 360 SL on Biochemical Parameters

The results revealed a change in serum protein concentrations and high enzymatic activity. Indeed, transaminase (ALAT and ASAT) assays revealed a significant increase in female rats treated with Kalach 360 SL compared to controls. Transaminases are enzymes located in several tissues, namely the liver, heart, and muscle. They are synthesized in the cytoplasm of the cells of these organs and released into the plasma in high quantities when these organs are damaged (Ruhl, 2012) [10]. According to Kuzu et al. (2012) [11], this increase in the serum concentration of transaminases would be due to a strong release of enzymes into the blood. The displacement of these enzymes could be caused by an inflammatory reaction or necrosis of the liver tissue (Kuzu., 2012, Chiali et *al*., 2013) [11-8]. Indeed, the high activity of these enzymes (transaminases) could be caused by destructive changes in liver cells by the pesticide, inducing tissue necrosis (El-Demerdash et *al*., 2012) [12]. This necrosis then promotes cell membrane permeability, hence the movement of enzymes into the plasma (Chiali et *al*., 2013) [8]. This membrane permeability in the liver could explain the increase in the mass of this organ. Exposure to Kalach 360 SL for 60 days would considerably aggravate the deleterious effects of the active ingredient of this herbicide. A study conducted by Yahia (2012) [3] revealed an increase in the serum concentration of ASAT and ALAT in rats after exposure to mancozeb (fungicide) at doses of 250, 500 and 1000 mg/kg of body weight.

At the same time, we observed a significant decrease in the serum concentration of Total Protein in the groups treated at doses of 500 and 1000 mg/kg of body mass of Glyphosate. Proteins are molecules synthesized by the body to accomplish very specific tasks. Therefore, the serum level of these molecules depends on certain factors, namely nutritional, hepatic, renal and hormonal. Thus, the level of Total Protein in the blood provides information on the general state of certain important organs such as the liver (Limidi and Hyde., 2003) [13]. This decrease confirms the hypothesis that the increase in the serum concentration of transaminases would be due to liver damage. According to two authors, this decrease would be due to a hepatic disorder which results in the disruption of the synthesis of these proteins (Eissa and Zidane, 2009) [14]. Studies of pesticide exposure have also resulted in a significant decrease in serum protein levels, reaching this conclusion (Kalender et *al*., 2005) [15].

The results reveal a significant increase in serum cholesterol concentrations in rats treated with glyphosate at doses of 250, 500, and 1000 mg/kg body weight. Cholesterol is a lipid of the sterol family that plays a central role in many biochemical processes (Benzécri et *al*., 1985) [16]. Increased cholesterol concentrations are linked to an increase in the cardiovascular disease index (Law et *al*., 2003) [17]. Analysis of these different renal parameters revealed a significant increase in Urea concentration in groups treated with doses of 250, 500 and 1000 mg/kg body weight of Glyphosate with Kalach 360 SL compared to the control group. Urea and Creatinine concentration is an important marker for the diagnosis of renal function (Gnanamani et *al*., 2008; Mukinda et *al*., 2010) [18 - 19]. Urea is filtered by the renal glomerulus and part is partially reabsorbed by the tubules. The increase in its concentration in the blood (uremia) is thought to be due to a disruption of renal function (Traynor et *al*., 2006) [20]. Long-term exposure to Kalach 360 SL is thought to have a significant impact on the renal function of female rats. The results also showed an increase in the serum concentration of Creatinine in the groups treated with doses of 500 and 1000 mg/kg of body mass of Glyphosate compared to the control group. Creatinine is a product resulting from the dehydration of creatine. This product is secreted into the blood and then eliminated in the urine. The high concentration of Creatinine observed in the result would therefore be related to the disruption of renal function (Traynor et *al*., 2006) [20]. Kalach 360 SL would impact the liver and kidney function of female rats.

5.3. Effects of Kalach 360 SL on Hormonal Parameters

The data revealed a disruption in hormone concentrations. This disruption is characterized by a significant decrease in serum progesterone and FSH concentrations, and an increase in serum estradiol concentrations. A significant decrease in serum FSH concentrations in rats was observed after 60 days of daily administration of Kalach 360 SL (360 g/L of glyphosate). Exposure to Kalach 360 SL is thought to impact the production of this hormone. A study by Zidan (2009) [21] reveals disruption of the acetylcholinesterase enzyme as being responsible for this phenomenon. Indeed, organophosphate mixtures destabilize the normal process of this enzyme, leading to an increase in acetylcholinesterase in the brain. An increase in the level of this enzyme in the brain would cause a reduction in FSH levels (Zidan, 2009) [21]. According to Ngoula et *al*. (2007) [22], carbamate (an insecticide) would be able to disrupt the activity of

acetylcholinesterase and reduce nerve impulses. This action would modify the release of FSH (Mathur and D'Cruz, 2011) [23].

Data on female rats treated with Kalach 360 SL (360 g/l of glyphosate) revealed a decrease in serum progesterone concentrations. The ovary, in addition to gamete production, has a triple regulatory role that makes it an essential organ for female reproduction through the action of ovarian hormones secreted by the follicles (Chalmey, 2013) [24]. Progesterone is a hormone produced by the corpus luteum established after ovulation (Chalmey, 2013) [24]. It promotes the acquisition of progesterone receptors in the ovary, which are carried by the granulosa cells of preovulatory follicles in response to the LH surge (Akison and Robker, 2012) [25]. At the same time, the released progesterone acts on the uterus through endometrial proliferation (Groothuis et *al*., 2007) [26]. Therefore, the low level of this hormone observed in our study would demonstrate the negative impact of Kalach 360 SL (360 g/L of Glyphosate) on the reproductive function of female rats.

On the other hand, an increase in serum estradiol concentration was observed in female rats treated with different doses of Kalach 360 SL (360 g/L of Glyphosate). Exposure of female rats to different doses of Kalach 360 SL would result in an increase in serum estradiol concentration. This increase could be the result of the activation of an enzyme by the herbicide. Indeed, in vitro studies conducted with atrazine (Herbicide) have shown the induction of aromatase activity (the enzyme responsible for estrogen synthesis) in human cell lines (Sanderson et *al*., 2001) [27]. A study on human placental cells demonstrated that glyphosate disrupts aromatase activity (Richard et *al*., 2005) [28]. However, Kalach 360 SL contains glyphosate as the only active ingredient. Kalach 360 SL would disrupt the activity of the enzyme responsible for estrogen synthesis (aromatase) 4-4- Impact of Kalach 360 SL on Liver Histology

The results of the histological examination of the liver of female rats treated for 60 days show a disorganization of the liver tissue architecture. This disruption is believed to be the result of necrosis and vacuolation of hepatocytes in rats exposed to Kalach 360 SL. According to Yahia (2016) [3], all these observed changes are due to the accumulation of high quantities of Mancozeb (fungicide) in hepatocytes. According to some authors, lipoprotein oxidation disorders and excess oxidative stress lead to necrosis in hepatocytes (Ritziu et *al*., 2010) [29]. Indeed, reactive oxygen species (ROS) or reactive oxygen species (ROS) are chemical species produced by the insecticide (Chlorpyrifos). They are often implicated in the occurrence of oxidative damage in liver tissue (Heikal et *al*., 2012) [30]. According to this study, Chlorpyrifos, in addition to increasing the level of ERO, would lead to a decrease in the antioxidant activity of the enzymes superoxide disputase (SOD), catalase (CAT) and glutathione peroxidase (Gpx) which would cause liver damage (Heikal et *al*., 2012) [30]. Ikhlef (2017) [31], indicated several changes after treatment with a mixture of Roundup and Pyrical (Glyphosate and Chlorpyrifos). Congestion of the centrilobular veins and necrosis (cell swelling) in certain regions of the liver were observed. Mamum et *al*. (2014) [32] proved that the formation of vacuoles in hepatocytes and congestion of blood vessels after exposure to carbamates would be due to hemorrhage. The results of histological observations similar to ours have been listed by several researchers (Choudhary et *al*., 2003; Caglar and Kolankaya, 2008) [32-34].

5.4. Effects of Kalach 360 SL on Kidney Histology

Histological examination of the kidneys of female rats revealed tissue damage characterized by lesions, necrosis, narrowing of certain ducts, and structural disorganization of the organ. Exposure of male rats to Kalach 360 SL is thought to cause structural disorganization of the kidney, which leads to functional disruption of the kidney. A study by Fortin et *al*. (2009) [35] on rats treated with lambda-cyhalothrin showed several hemorrhagic foci, tubular dilation in the proximal tubule, desquamation of tubular cells, and swelling of the tubules in the kidney. Ano et al. (2024) [36]. also revealed necrosis in the glomerular tissues, convoluted tubules, and glomerular tubules of the kidneys after treatment of rats with doses of 0.28, 0.56, and 1.12 mg/kg body weight of lambda-cyhalothrin.

5.5. Impact of Kalach 360 on the histology of the ovary and uterine horn

Histological studies of the ovary and uterine horn revealed structural disorganization of these organs. Histological examination of the ovaries revealed a decrease in the number of follicles and a significant increase in follicular atresia in rats treated with Kalach 360 SL, leading to degeneration of developing follicles and corpora lutea. According to some authors, pesticide-induced follicular atresia appears to be mediated by oxidative stress. Indeed, the increase in follicular atresia caused by malathion (organochlorine insecticide) is thought to be due to granulosa cell apoptosis under the effect of oxidative stress by increasing free radical formation (Bhardwaj and Saraf, 2014) [37]. Ullah et al. (2006) [38] observed histological changes in rabbit ovaries treated with a repeated dose of cypermethrin (insecticide) over a short period.

6. Conclusion

Exposure of female rats to the herbicide Kalach 360 SL (Glyphosate 360 mg/L) at doses of 250, 500, and 1000 mg/kg body weight of glyphosate for 60 days impacted several morphological and physiological parameters. The body mass gain was considerable in rats exposed to Kalach 360 SL for 60 days. In terms of relative organ masses, a significant increase in the liver and kidney was observed in female rats exposed to Kalach 360 SL for 60 days. In female rats, after 60 days of oral treatment at doses of 250, 500 and 1000 mg/kg body mass of Glyphosate, respectively, the results showed a decrease in the mass of the ovary and uterine horn. The biochemical study revealed a high production of ALAT, ASAT, Cholesterol, Urea and Creatinine in the treated female rats. In contrast, the production of total protein decreased in rats treated for 60 days. Hormonal assays revealed a decrease in serum FSH concentration, progesterone concentration and a significant increase in serum estradiol concentration in female rats exposed to Kalach 360 SL. Histological study at 60 days of exposure the histological study of the liver and kidney showed a very advanced structural disorganization. This damage observed within the liver and kidney confirms the variations in biochemical parameters as well as the variation in the relative masses of these organs. The histological study of the ovary and uterine horn revealed changes in the internal structure of the ovaries and uterine horn of female rats exposed to Kalach 360 SL. These results show the negative impact of Kalach 360 SL (Glyphosate 360g/l) on the health and reproduction of rats exposed over a long period. These results confirm the revealing reports of the effect of Glyphosate on mammalian reproduction and confirm that Glyphosate is an endocrine disruptor. In view of these results, we can affirm that the daily use of this herbicide by farmers would present dangers to health and reproductive function.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The animals were treated in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes. Every effort was made to minimize animal suffering and reduce the number of animals used.

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