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IMPACT OF GLYPHOSATE-BASED HERBICIDE ON THE REPRODUCTIVE SYSTEM AND SEMEN QUALITY OF WISTAR RATS

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SUMMARY

This study was conducted as part of the toxicity assessment of a glyphosate-based herbicide commonly used in Ivory Coast. The objective of this study was to highlight the effects of Kalach, a glyphosate-based herbicide, on the reproductive system of Wistar rats. For this purpose, 32 male rats were divided into four groups of eight rats each. The control group received distilled water. The other three groups received 0.1 ml, 0.2 ml, and 0.3 ml of Kalach, corresponding to 250, 500, and 1000 mg/kg/day, respectively. Glyphosate treatment was carried out for 60 days. At the end of treatment, the testes, epididymis, seminal vesicles, and prostate were removed and weighed. Testosterone, LH, and FSH levels in blood serum were measured. Sperm concentration, morphology, and motility were determined. Histological sections of the testes were also taken. The results revealed a significant reduction in the relative mass of the testes, epididymis, seminal vesicles, and prostate in the treated groups compared to the control group. Serum FSH and testosterone concentrations were also decreased in the treated rats. Another decrease in sperm concentration, motility, and normal shapes was also recorded. Histological damage to the seminiferous tubules, impaired spermatogenesis, and a reduction in the epithelial height of the seminiferous tubules were observed. It was concluded that doses of 250, 500, and 1000 mg/kg body weight of glyphosate, Kalach, had an impact on the reproductive system of rats. It has been recommended that glyphosate-based herbicides be used with great caution and strict safety measures. otherwise, use environmentally friendly control methods.

Keywords: Kalach, Rats, Testes, Hormones, Spermatic parameters

INTRODUCTION

The reproductive featuresin the various mammal species has been investigated in plenty of studies. The differences in these features are markedly wide and variable. In their study, Toppari *et al.* 1996 referred to sexual ambiguity, disturbances in the sex ratio, congenital anomalies, puberty, and hormonal imbalance in men. In most industrialized countries, it has been reported that problems related to testicular cancer in men have increased over the past 20 years (Auger *et al.,* 1995). Likewise, an increase in the incidence of hypospadias (Adami *et al.* 1994., Paulozzi *et al.,* 1997) and a significant decrease in sperm production have been reported (Carlsen *et al.* 1992; Auger *et al.,* 1995., Swann *et al.,* 1997). The precise reasons for these different remarks are unknown (Eustache and Auger 2003). However, the probable role of environmental pollutants is mentioned (Eustache and Auger 2003). In men and males of many species, the reproductive function is very vulnerable to environmental factors, whether physical or chemical (Amaral, 2002). The term pollutant is linked to the appearance in the environment of deleterious effects and which exerts a disruptive influence on the environment (Le mer, 2009). These are chemical compounds capable of having toxicity on the reproductive function. These compounds are capable of simulating, promoting or inhibiting the action of hormones, thus, disrupting reproductive functions (Amaral, 2002). Pesticides are substances intended to destroy unwanted organisms considered harmful. Pesticides are mainly used in agriculture aiming to eliminate weeds (herbicides) and fighting harmful insects (insecticides) or the development of pathogenic fungi (fungicides)(Giroux, I2004). The quantity of pesticides applied each year to crops across the planet is estimated at 2.5 million tones (Van der Werf, 1996). However, the quantity of pesticides that directly comes into contact with the target undesirable organisms is very small (Van der Werf, 1996). According to researchers, approximately 0.3% of the quantity of pesticides comes into contact with the targeted organisms, which means that 99.7% of the substances spilled end up in the environment (Pimentel, 1995). Pesticides used to combat harmful organisms end up in the environment where they risk causing contamination (Pimentel, 1995). Herbicides are active substances or products formulated to kill plants. Among these herbicides is Kalach 360 SL, a Glyphosate-based herbicide distributed by Callivoire. It is used in Ivory Coast in the agricultural sector. Kalach 360 SL is effective on weeds and improves crop yield. It is a less toxic substance with LD50 greater than 2000 mg/kg b.w. This is why Arysta Life Science and UPL classify it as a low-risk product. Although less dangerous, this substance would develop certain notable effects.

Thus, the evaluation of the potential toxicological risks of Kalach on human health, in particular on reproductive health, constitutes a societal concern (Choudhary et Joshi 2003). The current study is a part of evaluation of the toxicity of a Glyphosate-based herbicide. The objective of this study is to evaluate the effects of Kalach 360 SL (a Glyphosate-based herbicide) on the reproductive system of the wistar rat.

MATERIAL AND METHODS

Animal and chemical materials:

Thirty two (32) adult male rats weighing between 120g and 130g were used in this study. The rats were brought from the ENS vivarium in Abidjan. They were fed corn, bread and a formulation based on soy (20%) + fish (35%) + corn (45%). They were raised in polyethylene cages lined with mesh wood shavings bedding with free access to water and food. The cages are cleaned every two days. The chemical substance used is Kalach 360 SL (Glyphosate 360 g/l). This herbicide is distributed in Ivory Coast by Callivoirewho is a subsidiary of Unité Phosphorus Limited, a global player in the plant protection industry. In Ivory Coast, Kalach 360 SL is used on a wide range of crops (tomato, carrot, cabbage, cucumber, rice, oil palm, cotton). It is one of the most popular herbicides among pesticides used by Ivorian farmers because of its effectiveness on weeds and its non-toxicity on crops.

Treatment of rats:

According to Aymeric (Aymeric, 2016), the LD50 of Glyphosate is greater than 8000 mg/kg of body weight. Based on the original Guideline 408, it was adopted in 1998, 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 mc. Four batches of eight rats each were formed. Rats are treated daily by gavage for 60 days. Each batch received 1ml of the corresponding preparation individually and daily. So;

- ✓ the Control Lot received distilled water;
- ✓ batch 1 received 250 mg/kg of m.c. Glyphosate, i.e. 0.1 ml of Kalach + 0.9 ml of distilled water;
- ✓ batch 2 received 500 mg/kg of m.c. Glyphosate, i.e. 0.2 ml of Kalach + 0.8 ml of distilled water;
- ✓ batch 3 received 1000mg/kg of m.c. Glyphosate, i.e. 0.3ml of Kalach + 0.7ml of distilled water.

Sample collection:

After 60 days of treatment, the animals were sacrificed after anesthesia, the blood of the animals was collected before sacrifice. Blood collection was carried out using dry tubes (without anticoagulant). The blood in the dry tubes was used for hormonal dosage. The testes, epididymis, seminal vesicle and prostate were removed by dissection. They were rinsed with 9% sodium chloride (NaCl), weighed and then preserved in 10% formalin.

Analysis of sperm parameters:

Sperm were collected according to the technique described by Ngoula *et al.*., 2007. To do this, a solution of 10 ml of 9% NaCl is prepared and incubated in a water bath at 36°C. Before sacrifice, the tail of the rat's epididymis is removed then dilacerated and macerated in the Na CL solution prepared beforehand. Sperm concentration was determined using the Malassez cell. To do this, a drop of the macerate from the tail of the epididymis is taken and placed on the Malassez blade. After covering the slide with a coverslip, the observation was made under an optical microscope (Olympus CX31RBSF, Philippines) at magnification (X 100). The sperm concentration is calculated according to the following formula: Concentration (SPZ 10 /ml) = (D.V.n) / N
D: dilution coefficient (50)., V: volume of the Malassez cell., n: the number of spermatozoa counted in 05 cells., N: the number of small cells

Sperm motility was determined by examining the previously prepared solution. A drop of this solution was taken and placed between slide and coverslip. The slide was then observed under a light microscope (Olympus CX31RBSF, Philippines) at 100 magnifications. The field of observation of mobile and immobile spermatozoa was divided into five. The percentage of mobile and immobile spermatozoa is determined by the following formula:

$$\% \text{ Motile sperm} = \frac{\text{Total number of motile sperm}}{\text{Total sperm count}} \times 100$$

To determine the morphology of the spermatozoa, a drop of the previous solution is placed on a slide and spread using a coverslip. Then, the slide was stained using an eosin solution then observed under an optical microscope at 100 magnifications.

Hormonal analysis

The principle of dosing gonadal testosterone combined the competition method with final fluorescence detection (ELFA). To do this, boxes of 60 tests (VIDAS, BIOMERIEUX, France) containing all the immunological reaction reagents are used. The testosterone assay was carried out using the HITACHI 902, Automatic analyzer, Japan. The tested sample was taken and then transferred to the well containing testosterone derivative. Such that, there was a competition between the hormone present in the serum and the estradiol derivative of the conjugate. At the end of the test, the results were calculated by the controller in relation to a stored calibration curve and then printed. The principle of measuring pituitary hormones (LH and FSH) combined the sandwich enzymatic immune method with final fluorescence detection (ELFA). The dosage steps were the same as the gonadal hormones except that the antigen (hormone) was bounded on one hand to the immunoglobulins fixed on the cone and on the other hand to the conjugate, thus forming a “sandwich”.

Histopathology of the testis:

The testes were preserved in 10% formalin for 48 hours. They were then removed, rinsed and placed in

cassettes. The cassettes were labelled and introduced successively into four alcohol baths of increasing degree (80° in one hour, 90° in two hours and two baths of 100° of two hours each). After alcohol step, they were introduced into three successive toluene baths of one hour and two hours. Then the samples were directly immersed in the oven at 60°C successively in two baths of liquid paraffin for two (02) and three (03) hours. After impregnation, the cassettes were opened; the organs removed then placed in molds containing a fine quantity of paraffin. Then, the mold was covered by the cassette inside which the liquid paraffin was poured until full. After cooling and solidification, the microtome (Leica RM2125 RTS Germany) was used to produce sections with a thickness of four to ive µm. After this step, paraffin ribbons containing organ sections were obtained. The paraffin ribbons produced were placed on water heated to 40°C. They were mounted on object slides then the slides were placed in an oven between 58 and 60°C for 30 min for deparaffinization. The sections were deparaffinized in three successive toluene baths for 15 min each. After deparaffinization, they were passed through three successive alcohol baths of decreasing degree (100°, 95° and 75°) for five min each. At the end of the last bath, the sections were rinsed in distilled water. After switching to distilled water, they were immersed in a modified Harris hematoxylin bath (two to three min). The sections were rinsed in running water and immersed in a 3% eosin solution then rinsed in running water. Then, the sections were dehydrated using three successive alcohol baths of increasing degree (75°, 95°, 100°) for five min each. After staining, the sections were passed in two successive toluene baths for 15 min each. A drop of Eukitt was used to fix a glass coverslip on the histological section.

Statistical analysis of data:

Graph Pad Prism7 software was used for statistical analysis of the data. Results were presented as mean ± Standard Deviation (SD). The data were evaluated by the one-way ANOVA analysis method, followed by a Tukey multiple comparison test at the 5% threshold. The differences were considered significant at $P < 0.05$.

RESULTS

Relative mass of reproductive organs:

At the level of the testicles, the results showed that the rats from the groups treated with doses of 500 and 1000 mg/kg bw had the smallest testicular masses (0.968 ± 0.018 and 0.781 ± 0.012 g/100g bw) respectively. Statistical analysis showed a significant reduction in testicular mass in the groups treated with 500 and 1000 mg/kg bw compared to the control group ($P <0.05$).

For the batches treated with doses of 500 and 1000 mg/kg bw, the results revealed the smallest values of the epididymal mass (0.204 ± 0.015 and 0.136 ± 0.005 g/100g bw respectively. The statistical analysis revealed a significant reduction in the epididymal mass of the groups treated with 500 and 1000 mg/kg bw compared to the control group ($P <0.05$).

For the seminal vesicles, the results showed that the batches treated with doses of 250, 500 and 1000 mg/kg bw. were 0.489 ± 0.023, 0.453 ± 0.015 and 0.341 ± 0.020 g/100g bw respectively. Otherwise, the control batch showed seminal vesicles mass of 0.611 ± 0.016 g/100g of m.c. The statistical analysis showed a significant reduction in the mass of the seminal vesicles of the treated batches compared to the control batch ($P <0.05$).

At the level of the prostate, the results showed that the rats from the group treated with a dose of 1000 mg/kg of body weight had the smallest prostatic mass (0.119 ± 0.005 g/100g of body weight). The statistical analysis revealed a significant reduction ($P<0.05$) in the prostatic mass in this group compared to the control group (table I).

Table 1. Relative mass of the reproductive organs of rats

Glyphosate concentration (mg/kg m.c)	Relative masses of reproductive organs (g/100g body weight)			
	Testes	Epididymis	Seminal vesicles	Prostate
0	1.243±0.026	0.273±0.005	0.611±0.016	0.225±0,010
250	1.079±0.077	0.233±0.019	0.489±0.023*	0.179±0,014
500	0.968±0.018*	0.204±0.015*	0.453±0,015*	0.177±0.009
1000	0.781±0.012*	0.136±0.005*	0.341±0,020*	0.119±0.005*

Overall means of different reproductive organs (± SE) of treated and control rats (n = 8/batch)., Values in the same column with astrix are statistically significant ($P < 0.05$)., Values in the same column with no astrix are statistically non-significant ($P > 0.05$).

Spermatic parameters:

Sperm density:

The results showed that the highest dose (1000 mg/kg bw) had the lowest quantity of sperm while the control obtained the highest concentration. The statistical analysis shows that the sperm concentrations obtained in the batches treated at the respective doses of 250, 500 and 1000 mg/kg b.w. were significantly ($P< 0.05$) lower as compared to the control batch (Figure 1A).

Sperm morphology:

As shown in figure 1B, the respective dose batches of 250, 500 and 1000 mg/kg of Glyphosate m.chad higher rates of abnormal spermatozoa as compared to control batch.

Statistical analysis revealed significant increase in the rate of abnormal spermatozoa in the treated batches compared to the control batch (P<0.05).

Sperm motility:

Asillustratedin Figure 1C, alltreatment doses used resulted in lower rates of motile spermatozoa as compared to the control batch (P < 0.05).

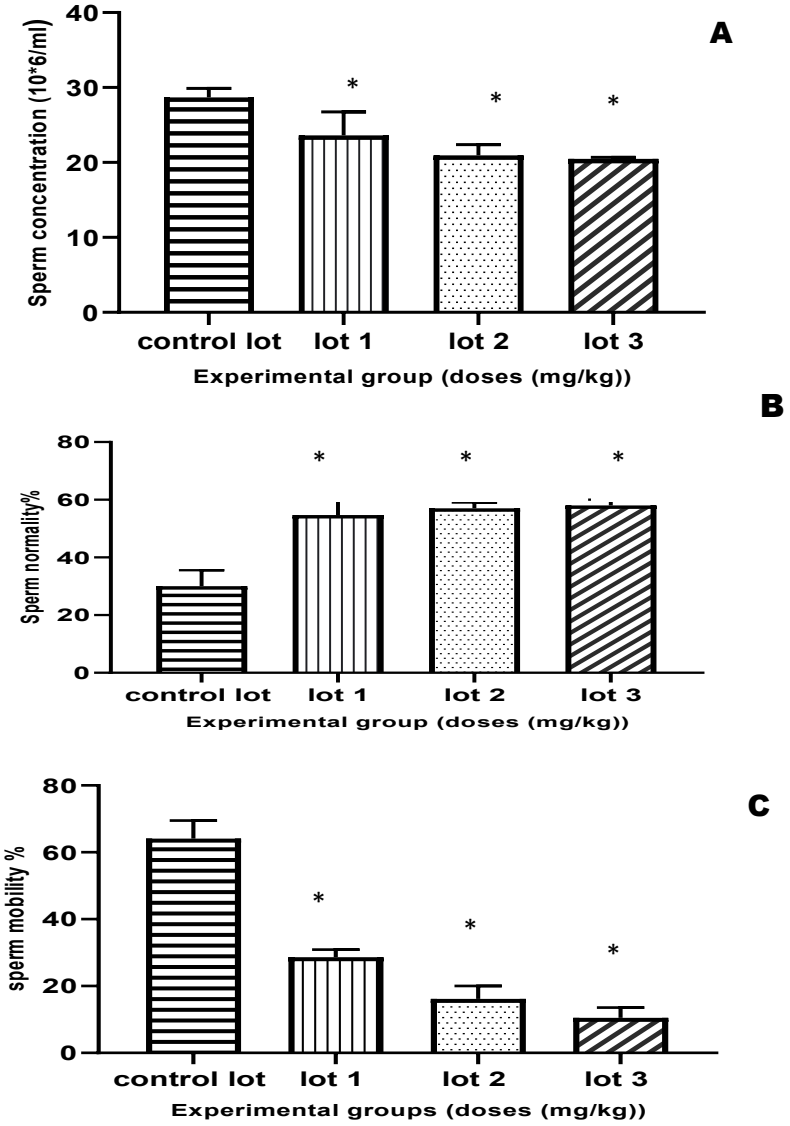

Figure 1. Sperm picture in the different treated and control rat groups, A = Sperm density; B = Sperm morphology; C = Sperm motility, * = Significant at P < 0.05.

Hormonal parameters:

Statistical analysis showed that all treatment batches had significant (P < 0.05) drop in serum FSH levelsas compared to the control batch (Figure 2B). On the other hand, the testosterone levels were significantly (P < 0.05) higher in all treated batches compared to the control batch (Figure 2C).

Histological findings of the testicles:

The histological observations of the testes of control rats showed normal structures; normal appearance of the seminiferous tubules; normal epithelial height of the tubules and normal spermatogenesis. Mature spermatozoa were also shown in the lumina of the seminiferous tubules (Figure 3 A). The Kalach treated rats showed apparent loss of spermatogenesis followed by a reduction in the number of germ cells, hence, widening of the lumina of the seminiferous tubules (Figure 3 B, C, D). Degeneration of Sertoli cells was observed in the batches treated with 1000 mg/ kg of m.c. Glyphosate. The presence of cellular debris in the lumena of the seminiferous tubules was also observed (Figure 3 B, C, D).

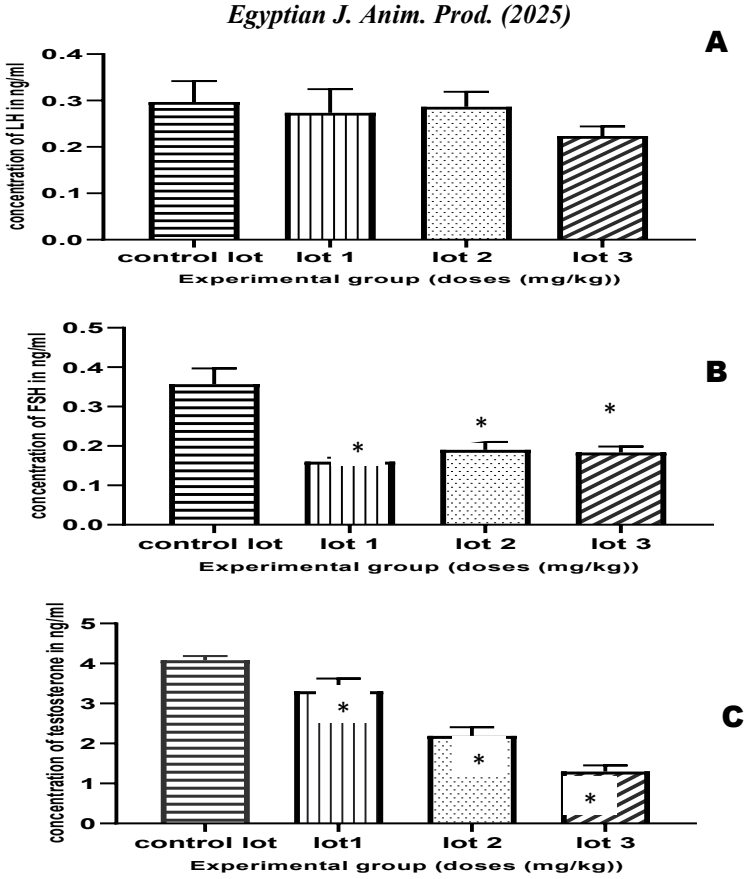

Figure 2. Impact of different doses of Glyphosate on serum hormonallevels in treated vs. untreated rats, * = Significant at P < 0.05. Significant.

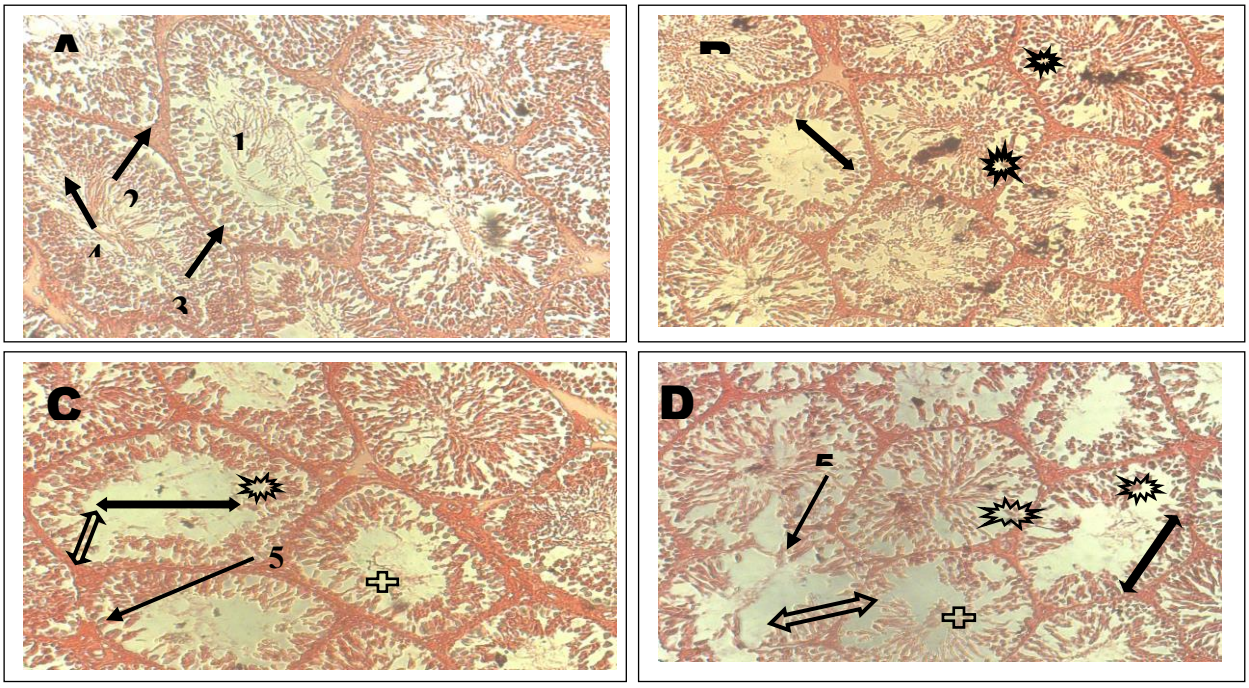

Figure 3. Histological observations of rat testes:
1: Lumen of the seminiferous tubules, 2: Interstitial space containing the Leydig cells, 3: Seminiferous tubule, 4: Germinal epithelium, 5: Lesion of the seminiferous tubules. Loss of spermatogenesis Enlargement of the lumen of the seminiferous tubule , Cellular debris, Degenerated and injured epithelium.
A: control rat; B: Treated rat with 250 mg/kg bw; C: Treated rat with 500 mg/kg bw; D: Treated rat with 1000 mg/kg bw.

DISCUSSION

After 60 days of Kalach administration, the rats started to show reduction in the testicular mass (compared to control rats). The decrease in the testicular mass of the treated rats could be due to cellular atrophy of the gonads. According to a study by Razi *et al.,* 2012, exposure of rats to a dose of 125 mg/kg of Glyphosate for 10, 20, 30 and 40 days led to a significant reduction in testicular weight.Penna-videau *et al.,* 2012, reported that cellular atrophy of the testes was the basis of reduction in testicular mass in treated rats.Yahia 2016also showed that the testes of rats decreased following exposure to mancozeb for 8 weeks.

In addition, the results showed a decrease in the relative mass of the epididymides of treated rats. It is well known that the epididymis is the storage and maturation site for spermatozoa after the completion of spermatogenesis process in the testes. The decrease in its mass could indicate the adverse effect of Kalach on the epididymides. According to Klinefelter *et al.*2002, the reduction in the density of spermatozoa in the epididymis may be the cause of the drop in its mass. Espinoza-Navarro and Bustos-Obregon2014 also observed a decrease in the relative mass of the epididymis following exposure of rats to malathion at a dose of 170 m g/kg for 13 days.

This study showed that exposure of rats to Kalach SL, resulted in a decrease in the relative mass of seminal vesicles of the treated rats. Kenfack *et al.,* 2007 mentioned that the decrease in the testicular mass of the treated rats could be a reasonable cause for the decrease in the seminal vesicles mass. Thibault and Levasseur, (2001) showed that the development of the accessory glands and the extra-testicular genital tract is under the influence of testicular androgens

Another decrease in the relative mass of the prostate gland was also observed in animals exposed to Kalach. The prostate is made up of three pairs of glands including: dorsal lobe, ventral lobe and lateral lobe. In another study, the prostate of the rats showed prominentatrophy, inflammation and disturbance due to the treatment (McNeal 1981).

Sperm concentration was also found to decrease in sixty-days Kalach treated rats. According to Yousef *et al.,* 1995, the effects of glyphosate on sperm quality could be due to a direct cytotoxic effect on spermatogenesis and/or an indirect cytotoxic effect via the hypothalamic-pituitary testicular axis, which control the efficiency of spermatogenesis. Intheir study, Romano *et al.,* 2012 showed considerable reduction in the number of germ cells in male rats treated with 5, 50 and 250 mg/kg bw of Roundup (480g/l Glyphosate) by gavage for four weeks.

Romano *et al.,* 2012 in the study, a significant increase in the rate of abnormal spermatozoa in Kalach treated rats was also recorded. Exposing rats to Kalach would increase the rate of abnormal sperm.Theyalso provided evidence of increased levels of dead sperm. They reported that exposure of rats to respective doses of 5, 50 and 250 mg/kg bw of Roundup (480g/l of Glyphosate) had significant impact on sperm morphology.

In our study, sperm motility revealed a significant decrease in all rats treated with Kalach. According to Gupta *et al.,* 1992, exposure of rats to carbamate visibly impacted the mobility of spermatozoa, thus, affecting their fertilizing capacity. Other authors have also shown a reduction in the number of motile spermatozoa as well as an increase in the percentage of dead and abnormal spermatozoa after chronic exposure to a mixture of insecticides (Marmol-Maneiro *et al.,* 2003).

The FSH levels decreased in Kalach treated rats. This could be attributed to the adverse effect of Kalach on the pituitary gland. Zidan, 2009 attributed the decline in FSH levels to the disruption of Acetyle cholinesterase in response to Kalach treatment. This is to say that organophosphates may destabilize the normal process of this enzyme, leading to an increase in Acetylcholinesterase in the brain. This enzyme elevation causes a reduction in the levels of LH and FSH and subsequently results in reduction in the testosterone levels (Zidan, 2009). According toNgoula *et al.*2007 carbamate is capable of disrupting the activity of acetylecholinesterase, thus, reducing nerve impulses. This action would modify the release of pituitary hormones (LH and FSH), which would reduce sperm production in the testes (Jorssraei *et al.,* 2010, Mathurand D'Cruz, 2011).

Another decrease in serum testosterone concentration in male rats was also observed after Kalach exposure. Testosterone plays a vital role in spermatogenesis. The low concentration of this hormone would be a propable cause of the low sperm concentration. This could be explained by an alteration in the enzymatic activity responsible for spermatogenesis (Choudhary and Joshi 2003; Kenfack *et al,* 2007). For certain authors, the decrease in the concentration of testosterone in rats exposed to methoxychlor and 2,2-bis(p-hydroxyphenyl) would be due to an inhibition of esterase activity in Leydig cells which results in a decrease in the synthesis of testosterone (Uzumcu *et al,* 2004; Recio-Vega *et al.,* 2008). Zidan, 2009, reported that the increase in the level of Acetylcholinesterase in the brain would cause a reduction in the levels of LH and FSH, which in turn, reduces secretion of testosterone. The impaired testosterone secretion would also be caused by the disruption of the StAR protein (Steroidogenic Acute Regulatory Protein). Several authors reported that the commercial formulation of Glyphosate (Roundup) can disrupt steroidogenesis by reducing the level of StAR proteins of Leydig cells, which no longer allows the production of testosterone (Walsh *et al.*., 2000). Another authors observed a drop in testosterone concentration after treatment with Glyphosate (Yousef *et al.,* 1995; Romano *et al,*2012). Clair *et al.,* 2012). They noted a decrease in testosterone after in vitro exposure of testicles to Glyphosate. They exposed adult rats to endosulfan for ten weeks.

They observed a considerable decrease in serum testosterone concentration. According to these authors, this observation would be the result of an inhibition of testicular function, thus, influencing sperm parameters (Auger *et al.,* 1995; Modaresi and Seif, 2011).

The histopathological oservations showed changes in the internal structure of the testicles in treated rats. These changes included alterations in the process of spermatogenesis and degeneration of Leydig cells. Widening of the luminal diameters of the seminiferous tubules (indicating reduction in the epithelial height) were accompanied by destruction of the basal membranes of the tubules and loss of Sertoli cells. According to Ikhlef and Yahiaoui 2017, exposure of rats to a mixture of Roundup and Pyrical (Glyphosate and Chlorpyrifos) at doses of 21.66 and 32 mg/kg bwresulted in impaired spermatogenesis and lesionsin the tubules. In their research, Romano *et al.* 2012, studied the effect of Glyphosate on the testicular tissues of rats treated with 5, 50 and 250 mg/kg bw for four weeks. They found drastic alterations in the seminiferous tubules (Including an increase in the lumen diameters of the tubules accompanied by a reduction in the number of germ cells. The results of this study comesin complete agreement with those of Yahia, 2016 who reported a significant loss of spermatogenesis and an alteration in the seminiferous tubes of rats after exposure to Mancozeb for eight weeks.

CONCLUSION

Exposure of rats to the Kalach SL herbicide (Glyphosate 360 g/L) caused significant lesionsto their reproductive system. A sixty-days oral administration of 250, 500 and 1000 mg/kg of Glyphosate to rats, resulted in a reduction in their testicular mass, seminal vesicles, prostate gland and epididymides. A decrease in sperm concentration, sperm motility and an increase in abnormal sperm were also observed. The hormonal assay revealed a decrease in serum FSH and testosterone levels. The Histological observations of the testes showed changes in the internal structure of the seminiferous tubules of treated rats. The results of this study have confirmed the hazardous effect of KalachSL, a Glyphosate-based herbicide on the reproduction of rats. Such that, glyphosate-based herbicides should be used with great caution and strict safety measures.

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تأثير مبيد أعشاب قائم على الجليفوسات على الجهاز التناسلي وجودة السائل المنوي لجرذان ويستار

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أجريت هذه الدراسة كجزء من تقييم سمية مبيد أعشاب قائم على الجليفوسات شائع الإستخدام في ساحل العاج. كان الهدف من هذه الدراسة هو تسليط الضوء على آثار كالاش، وهو مبيد أعشاب قائم على الجليفوسات، على الجهاز التناسلي لجرذان ويستار. ولهذا الغرض، تم تقسيم ٣٢ جرذًا ذكرًا إلى أربع مجموعات تضم كل منها ثمانية جرذان. تلقت المجموعة الكنترول ماءً مقطراً. تلقت المجموعات الثلاث الأخرى ٠,١ مل و٠,٢ مل و٠,٣ مل من كالاش، أي ما يعادل ٢٥٠ و٥٠٠ و١٠٠٠ ملغم/كغم/يوم على التوالي. استمر العلاج بالجليفوسات لمدة ٦٠ يومًا. في نهاية العلاج، تم إستئصال الخصيتين والبربخ والحويصلات المنوية والبروستاتا ووزنهم. تم قياس مستويات هرمون والتستيرون ، LH، FSH في مصل الدم. تم تحديد تركيز الحيوانات المنوية وشكلها وحركتها. أُخذت مقاطع نسيجية من الخصيتين. أظهرت النتائج إنخفاضًا ملحوظًا في الكتلة النسبية للخصيتين والبربخ والحويصلات المنوية والبروستاتا في المجموعات المعالجة مقارنةً بالمجموعة الكنترول. كما انخفضت تركيزات FSH والتستيرون في المصل لدى الفئران المعالجة. وسُجل أيضاً انخفاض آخر في تركيز الحيوانات المنوية وحركتها وأشكالها الطبيعية. ولوحظ تلف نسيجي في الأنابيب المنوية، وضعف في تكوين الحيوانات المنوية، وإنخفاض في إرتفاع الخلايا للأنابيب المنوية. وخلصت الدراسة إلى أن جرعات ٢٥٠ و٥٠٠ و١٠٠٠ ملغم/كغم من وزن الجسم من الجليفوسات، كالاش، كان لها تأثير على الجهاز التناسلي للفئران. وقد أُوصي باستخدام مبيدات الأعشاب التي تحتوي على الغليفوسات بحذر شديد وإجراءات سلامة صارمة. وإلا، يتم إستخدم طرق مكافحة صديقة للبيئة.