Mancozeb has inhibited the reproduction mechanism in male domestic pigeons (*Columba livia domestica*) and altered hepatic function

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Info. Article

Historique de l'article

Reçu le 06/12/2024
Révisé le 26/01/2025
Accépté le 13/04/2025

Keywords:

Mancozeb, pigeon, saisonal reproduction, photoperiod, biochemical markers.

ABSTRACT

Pesticides are toxic chemicals, very much used in agriculture in order to increase the agricultural outputs. These molecules can affect reproductive function of animals. This study aimed to the toxicity of the fungicide mancozèb on certain blood biochemical markers and seasonal reproduction of male domestic pigeons (Columba livia domestica) under long photoperiod (20L: 04D). The fungicide was orally administered at 2 and 5 g/l (doses used in agriculture). The obtained results revealed that under a long photoperiod, the sexual activity lasted only 04 weeks. In addition, mancozeb administration induced gonadic regression, delayed the refractory phase, provoked a hyperglycemia, a hyperlipidemia, an elevation in the activity of alkaline phosphatase and aminotransferase, and a reduction in creatinine level. The toxic effects of mancozeb was apparent in higher doses. To conclude, mancozeb has inhibited the reproduction mechanism in male pigeons and altered hepatic function.

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1. INTRODUCTION

Mancozeb is a fungicide widely applied in the fields in order to eradicate plant pests. It is a famous product due to its low toxicity on non-target organisms, with a weak acute toxicity [1]. Furthermore, it is known to be lethal to nematodes at agricultural concentrations, while the sub lethal doses had affected nervous system, heat shock reactions and larval growth [2]. Mancozeb has also been shown to induce significant increases in reactive oxygen species (ROS) and cause mitochondrial inhibition in *in vitro* studies. It has been repported that Mancozeb have heamatotological and biochemical effects and thyroid toxicity [3].

As for reproductive toxicity in general, it is difficult to ascertain whether the chemicals are harmful to such physiological function. Mancozeb impairs female reproductive performance with blastomeric apoptosis in embryos at very low concentrations [4]. Mancozeb was demonstrated to alter implantation in mice [4]. Sexual organs were shown to be reduced in rats exposed to mancozeb, while long period treatment by mancozeb has disrupted spermatogenesis, it raised testicular lipid levels, degeneration in somniferous and epididymal tubules with loss of sperms. Biochemical changes of gonads were observed in male rats after chronic exposure to mancozeb [5].

In view of paucity of information on reproductive system, the present study has been undertaken to know the possible effects of mancozeb on reproductive cycle and some biochemical markers of domestic male pigeon (*Columba livia domestica*).

2. MATERIALS AND METHODS

2.1. Chemicals

Mancozeb or Zn, Mn-ethylene bisdithiocarbamate, a carbamate fungicide (bayer France) was diluted with drinking water of pigeons to obtain the required concentrations, which then given to birds.

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2.2. Animals

Male pigeon (*Columba livia domesica*), which had been captured locally, were kept in light-controlled rooms in metal cages measuring (100 x100x100) cm, with six birds per cage. They had been under a natural photoperiod, ambient temperature and humidity of 20±2 °C and 55% ± 4, respectively for 15 days. Food (chick crumbs) and water were provided *ad libitium*. Eighteen pigeons were divided equally to three groups, in which the first group was used as a control, but the second one has received 5 g/l of mancoeb (tech 75% purity); a concentration used in agriculture. However, the third group was given 2 g/l of mancozeb. All groups were held under artificial photoperiod of (20L: 4D) using electric horologe of an intensity of 72 watts. Hence, water containing mancozeb was renewed every 48h.

2.3. Laparotomy and blood sampling

Gonadal development was assessed by laparotomy at intervals of approximately 15 days. The gonads were examined through a small incision in the body wall between the last two ribs, after anesthetizing the incision with viscous lidocain. And the dimensions of the left testis measured to the nearest 0.5 mm. Testicular volume was calculated V= 4/3 π a2 b; where a is half the width and b is half the length (long axis).
Blood samples were obtained by pricking a superficial wing vein and collecting approximately 1 ml blood into heparinised tubes.

2.4. Analytical procedures

The plasma enzyme activities of alanine aminotransferase (ALT), plasma alkaline phosphatase (ALP), creatinine were measured by using commercially diagnostic kits obtained from Randox Laboratories (Ardmore, Northern Ireland, UK) using an automate CX9 (BECKMAN, Brea, CA, USA) as well as for glucose (kits supplied by Diamond Diagnostic).

The total cholesterol (TC) and triglycerides (TG) were analyzed by the enzymatic colorimetric methods (Randox reagent). TC was measured according to the enzymatic endpoint cholesterol oxidase-phenol aminophenazone method. TG were measured according to the glucose oxidase-phenol-aminophenazone method after enzymatic hydrolysis by lipases.

2.5. Statistical treatment

Data were presented as mean values ± SEM by using ANOVA, followed by Student's t test to assess significant differences among treated groups. All statistical analyses were performed using Minitab version 16.The significance was defined as $p \leq 0.01$.

3. RESULTS

Changes in testicular size

Testicular volumes were presented in fig 1. At the beginning of the experiment, all birds had mean testicular size of 188.3±36 mm^3. Control birds that were kept on 20L: 4D; long photoperiod throughout the experiment, all maintained fully reproductive cycle, characterized by significant ($p \leq 0.01$) increase in the volume of their testes at week 4, followed by spontaneous gonadal regression, with testes reaching a minimal size of 43.21 ± 8.4 mm^3 ($p \leq 0.05$) by week 8 of the experiment. No significant changes were observed testicular volume during the period of study. Though, treated pigeons have higher testicular volume compared to the control at the last week.

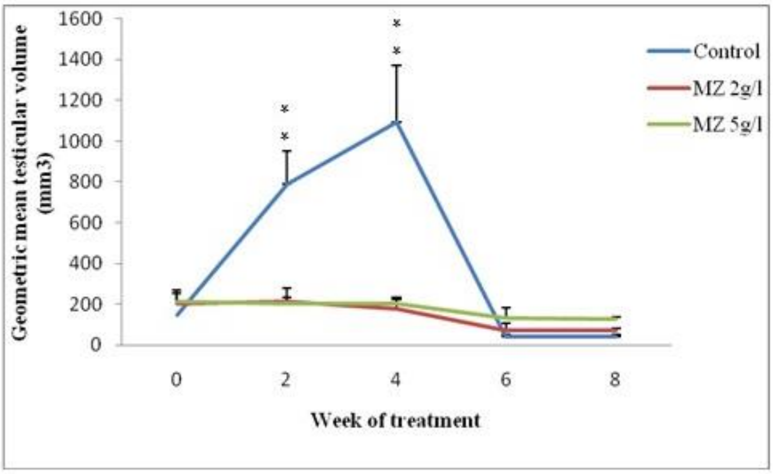

Fig 1: Change in testicular volume (mm3) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean±SEM (n=6). Significant differences at $p \leq 0.05$ and $p \leq 0.01$ (ANOVA followed by Student's t test).

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Changes in biochemical markers

Alanine amino transferase

The changes in alanine amino transferase (ALT), of males' pigeons (Columba livia domestica) held under 20 L: 4 D and treated with mancozeb are given in Fig 2. Treatment of domestic pigeon with mancozeb induced a significant increase ($p \leq 0.05$) in ALT (27.45, 30.73 and 34.54 U/l for the control, 2 and 5g/l mancozeb, respectively) at the 4th week of experiment.

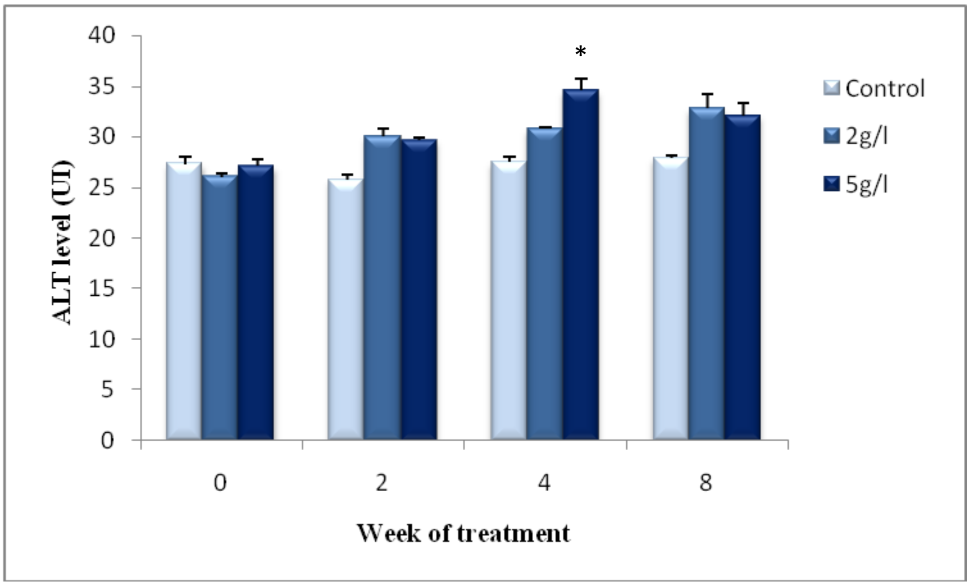

Fig 2: Change in (ALT) level (UI/L) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean±SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Alkaline phosphatase

Results relative to changes in alkaline phosphatase (ALP) are summarized in Fig 3. Obtained results revealed that treatment by mancozeb had produce a significant increase ($p \leq 0.01$) in enzymatic activity of alkaline phosphatase (ALP) until the 2nd week of experience, where we have recorded a mean level of 147.22, 599.75 and 703 UI/L for controls 2 and 5g/l mancozeb, respectively. It is important to note that control pigeons hadn't shown any changes in (ALP) through the experiment.

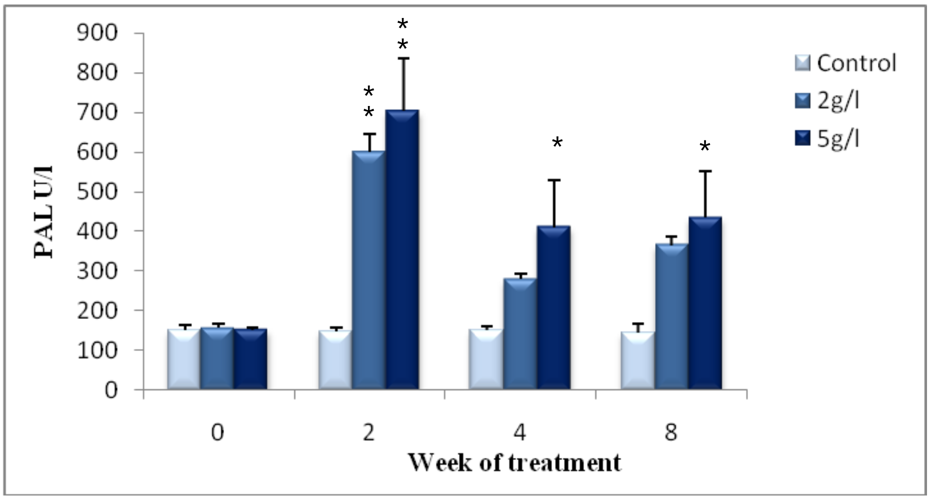

Fig 3: Change in (PAL) level (UI/L) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean±SEM (n=6). Significant differences at $p \leq 0.05$ and $p \leq 0.01$ (ANOVA followed by Student's t test).

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Glucose

The glucose concentration in blood of male pigeons (*Columba livia domestica*) placed under 20L: 4D and treated with mancozeb was significantly increased ($p \leq 0.05$) through the study from 3.28 g/l to 3.78 g/l in pigeon received 2g/l of mancozeb and from 3.34 to 4.86 g/l of blood glucose of pigeon treated with 5g/l of mancozeb. However, control pigeons have revealed a slight decrease in plasma glucose (Fig 4).

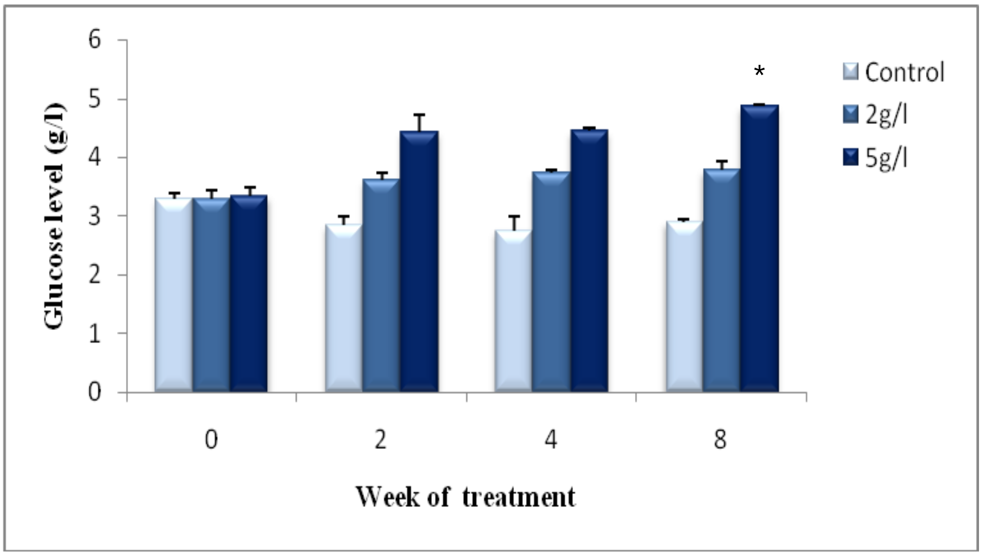

Fig 4: Change in plasma glucose (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Creatinine

The results obtained revealed a significant decrease ($p \leq 0.05$) in plasma creatinin. Whose we are recorded a value of 7.6 g/l of creatinin at the beginning of the study and 2.01 g/l at the end in 5 g/l of mancozeb treated. Control subject have reserved the same concentration of creatinin through the study (Fig 5).

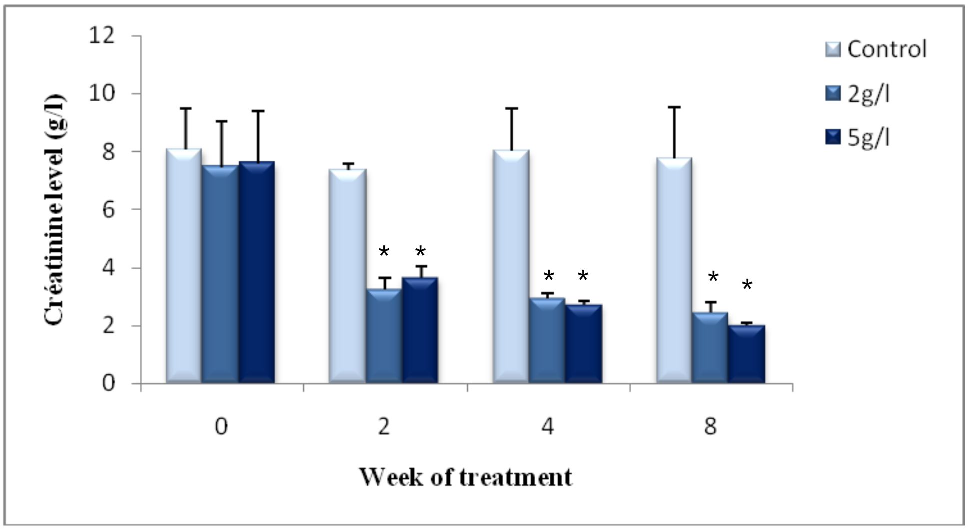

Fig 5: Change in plasma creatinine (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Cholesterol

The mean plasma cholesterol of control and treated male pigeons (Columba livia domestica) placed under long daily photoperiod 20L: 8D and orally treated with 2 and 5 g/ were shown in Fig 6. The treatment with mancozeb caused a significant ($p \leq 0.05$) elevation in plasma cholesterol till the 4th week of experiment in treated groups. While, controls had shown a non significant decrease in plasma cholesterol.

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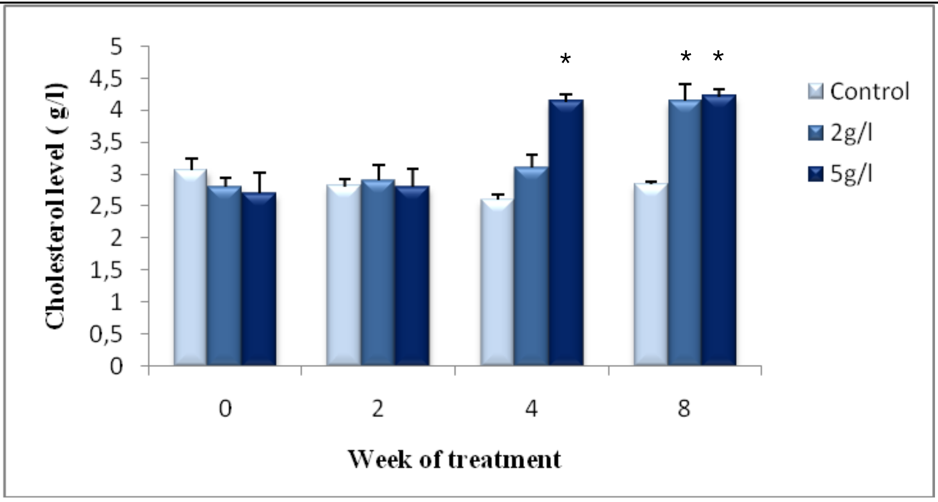

Fig 6: Change in plasma cholesterol (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Triglycerides

The results related to triglycerides recorded in this study are summarized in Fig 7. Data show a significant ($p \leq 0.05$) decrease in circulating triglycerides in mancozeb treated groups through the experiment. From 1.6 g/l to 4.3 g/l in 2g/l treated pigeons, and from 1.52 to 4.55 g/l among pigeons treated by the highest dose of mancozeb. There were no notable changes in triglycerides concentration in control subjects.

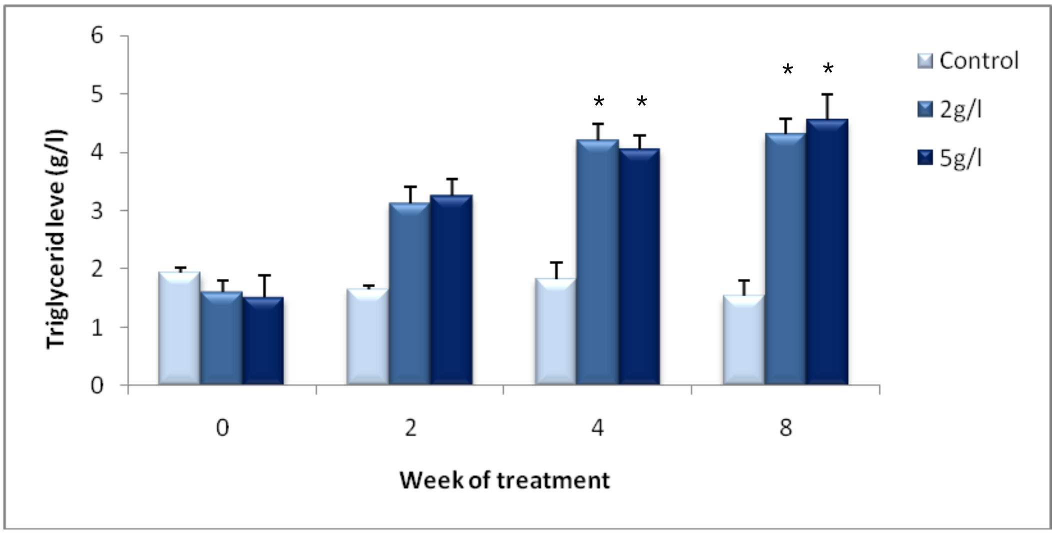

Fig 7: Change in plasma triglycerides (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

4. DISCUSSION

In birds, the gonadal growth and regression are highly seasonal and relate to environmental factors such as food availability and the photoperiod length. Therefore, the day length has been well defined as the regulator of different metabolic and reproductive activities in many avian species. It is known that birds use their extra-retinal photoreceptors, in combination with a circadian clock, to measure photoperiod [6]. Findings from this work indicate that under artificial photoperiod (20 L: 4D), birds maintained a fully reproductive cycle characterized by full mature testes at the 4th week, followed by spontaneous gonadal regression, as an increase in photoperiod rises the secretion rate of gonadotrophin-releasing hormone (GnRH) leads to increased LH and FSH, and then to steroid hormone synthesis [6]. However, the administration of mancozeb at a rate of 2 g/l and 5 g/l to male pigeons under long days, has inhibited the development of testes, and disturb their reproductive cycle.

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It has been recorded a lower means in testes sizes during the experiment in treated birds. Physiologically, it is possible that is attributed to the mechanism of photoperiod measurement. Therefore, they hadn't estimated the true photoperiod, and consequently all photoperiod would be regarded as being short [7].

On the other hand, it is possible that mancozeb have interfered directly on the testis as number of pesticides has showed testicular toxicity [7]. Study demonstrated that mice treated with mancozeb had a decreased testes weight and an inhibited spermatogenesis [5]. Other studies revealed a testicular atrophy with damaged germinal epithelium, accompanied with reduced sperm motility and viability in male adult pigeons exposed to maneb [8]. The toxic effect of pesticides on reproductive cycle of domestic pigeons (*Columba livia*) associated with a decreased testes size, reduced tubule size, and the reduced number of germ cells have been reported [9], [10].

Few studies have been carried out on the mechanisms of organometallic fungicide action on target organisms. However, many studies have been reported on the effects of heavy metals alone in a variety of organisms [11]. Exposure to most metals result in metal accumulation in certain tissues and organs of the exposed organisms. Chronic inhalation of high levels of Mn has been associated with a neurodegenerative disorder characterized by both central nervous system abnormalities and neuropsychiatric disturbances [12]. Zn is well known to accumulate in two particular organs, namely liver and kidney, where they may cause biochemical and histopathological changes [7].

In the present study, exposure to mancozeb resulted in biochemical disorders. Alanine amino transferase (ALT) and alkaline phosphatase (ALP) are primarily used to evaluate hepatic damage in clinical findings [13]. The results of this study demonstrated a notable elevation of plasma ALT and ALP activities of pigeons intoxicated by mancozeb fungicide. These findings agreed with that recorded in serum of thiram treated pigeons [13]. A higher activity of ALT in delthametrin, abamectin alone and combination treated pigeons [14]. This can be attributed to the tissue damage, especially hepatocytes [14]. Such findings are in-line with that reported earlier on the hepatocytes necrosis and aminotransferase activities elevation [13] and confirming that it may be attributed to liver injury [14].

Receiving mancozeb at dose of 2 and 5g/l led to an increase in plasma glucose in *Columba livia*., as a result of increasing catecholamines and corticosteroid hormones levels through the activation of gluconeogenesis pathway. Increased plasma glucose levels were recorded after exposing pigeons to thiram [13]. In this study plasma creatinine values were gradually decreasing with increasing dose of mancozeb. This decrease might be resulted from kidney functional impairments, which have been confirmed by tubular histopathological studies. Plasma cholesterol and triglycerides were significantly elevated, is possibly an indication of hepatocytes permeability [13]. Thus, the obstruction of bile duct can contribute in the decrease of cholesterol secretion through the bile, leading to its raise in the plasma [14].

5. CONCLUSION

In conclusion, our results indicate that under a long daily photoperiod of (20L: 4D), male domestic pigeon (*Columba livia domestica*) maintained a fully reproductive cycle characterized by full testicular maturity after one month, followed by spontaneous gonadal regression. However, the administration of mancozeb at a rate of 2 g/l and 5 g/l to male pigeons under long days, has inhibited the development of testes, and disturbed their reproductive cycle. Biochemical findings of this study suggest that mancozeb induced hepatotoxicity via an increase of alanine amino transferase and alkaline phosphatase activity, accompanied with hyperglycemia, hyperlipidemia and an elevation in creatinine level.

Acknowledgements

Authors are grateful to the PNR projects, Algiers for the financial support which was carried out within animal ecophysiology laboratory.

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