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The effect of the anticoagulant rodenticide "Brodifacoum" on the bioindicators parameters in male rabbit

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ABSTRACT

The effect of the anticoagulant rodenticide "Brodifacoum"on the bioindincators parameters inmale domestic rabbits was investigated.Animals were dividedintofourequal groups. The first one served as a control while the otherthree groups weredaily treated by Brodifacoumwithdoses of 0.01, 0.02 and 0.04 mg/kg of body weight. The rodenticide was administered per os for three weeks after which the animals were sacrificed.The obtained results reveal animportant decrease in body weight in treated groupsparticularly in those treated with the highest dose (0.04 mg/kg), whilean increase in the weight of liver was observed, associated with a marked reduction in the weight of spleen and kidney, compared to thecontrol one.Besides,levels of biochemical parameters were also significantly altered.The same observations were made concerning hematological parameters and plasmatic calcium levels in treated animals.Accordingly, it has been concluded thattreatment with Brodifacoum at the used doses and period may alter hematological and biochemical parameters in male rabbits.

Key words: Brodifacoum, anticoagulantrodenticide, rabbit, hematology, toxicity

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INTRODUCTION

To face the world growing population over the last decades, particularly in the developing nations, food production has been improved. Nonetheless, harvests are usually devastated by either insects or rodents. Rodents are also hosts for many human diseases, including plague, endemic rickettsiosis, leishmaniasis, spirochetosis, tularemia, leptospirosis, tick-borne encephalitis, and listeriosis [1, 2, 3]. Finally, rodents cause a variety of other damage, mainly by gnawing [4, 5, 6, 7]. Each year one-fifth of the world's food supply is consumed, contaminated, or condemned through contact with rodents [8].Therefore, rodent populations should somehow be controlled by limiting their access to food and harborage. Individual animals or small groups may be removed conveniently by trapping, although the need for poison use is still efficient for rodent control. The quest for the "better mousetrap" has been with us since before the Pharaohs [9].Pesticides cause more animal exposures than any other class of toxin. Pesticides include rodenticides, insecticides, avicides, fungicides, and herbicides [10, 11].Treatments with rodenticides are conducted regularly under specific regulations, either as plant protection products or as biocidal products[12]. Rodenticides are also used to help conserve native species on islands where rodents have been introduced, but adverse effects on non-target species have also been described [13, 14, 15, 16]. In addition, certain anticoagulants are used as drugs in human medicine [17].

The three main types of rodenticides currently in use are anticoagulants (such as Warfarin, Brodifacoum and Diphacinone), Bromethlin-containing compounds and Cholecalciferol [18].The most widely used rodenticides nowadays are anticoagulants which have an inhibitory action on the enzyme vitamin Kepoxide reductase,

responsible for recycling the vitamin K necessary for the production and activation of clotting factors II, VII, IX and X [19].

Modern rodenticides are much more toxic to rat and mice, but unfortunately are also more hazardous to non-target species such as children and domestic animals[9, 20].Spontaneous intoxications with anticoagulant rodenticides are reported in dogs [21, 22, 23, 24, 25, 26, 30], horses [31,32], cats [33,27], wild animals (deer, polecats, owls, eagles, falcons, ducks, martens, foxes, etc.) [34, 35, 36, 37,38, 39-42], and humans [43, 44, 45].

Compared to toxic substances in general, biochemical actions of rodenticides that have been studied in humans are not well documented. This makes it possible to assign them to groups that are meaningful not only chemically but in terms of biochemical lesions [17].

Brodifacoum, chemically named: (3- [3-(4' - bromobiphenyl-4-yl) -1, 2, 3, 4- tetrahydro-1-naphthyl] -4-hydroxycoumarin), is the most toxic anticoagulant rodenticide. It has been proposed for use against a rodent population [46]. The rodenticidal properties of Brodifacoum were described in 1976. It is an anticoagulant active against rats and mice including strains resistant to warfarin and other anticoagulants [47]. Brodifacoum has been classified by WHO in Class "Ia", extremely hazardous, based on acute oral LD_{50} of 0.2 mg/kg for rabbit [48-51]. It is also considered as a potent second-generation anticoagulant poison that is widely used in the eradication of vertebrate pests[51-56].Similarly, the product has generated a lot of scientific interests as shown by the number of publications illustrating its toxicity and efficacy in rodent's control [57, 58].There are few data available on repeated exposure of non-rodent species[59]. For instance, Gray *et al*. [60] investigated the toxicity of Brodifacoum, Difenacoum and Flocoumafen for barn owls fed poisoned mice.

The present investigation aims to evaluate the toxic effects of Brodifacoum on biochemical and hematological parameters on male rabbits.

MATERIALS AND METHODS

Animals and Treatment

In this present experiment, 28 male mature rabbits of *cuniculus lepus* were used. Animals were provided from local agricultural fields in Annaba. Animals were housed in steal cages (50cm x 60cm x53 cm) under natural conditions of temperature, humidity and photoperiod. After10 days of acclimatization period, they were divided into four groups of seven each. Food (a mixture of wheat grains, eggs (1:1) with some milk and water) were available *ad libitum*. At the start of the experiment, the animals weighted between 1500 to 2000 g. Body weight was recorded at 02 days interval throughout the experimental period. Brodifacoum was used *per os*. Individuals in group 1 served as control, while those of groups 2, 3 and 4receivedBrodifacoumat 0.01, 0.02 and 0.04 mg/kg of weight animal/day. At the end of the experimental period (03 weeks) the animals were sacrificed, blood was collected and desired organs (liver, kidney and spleen) were removed and weighted.

Hematological and biochemical parameters

The blood was collected in EDTA tubes for counts hematological study (white blood cells, red blood cells, hematocrit, hemoglobin and thrombocytes) by means of Coulter Counter Rubis. Collected plasma was used for determination of biochemical parameters: glucose as described [61];plasmatic level calcium was evaluated as described by Bauer [62].Triglycerides, cholesterol, urea, uric acid and creatinine levels were measured with the methods of [63].

Statistical analysis

The data are expressed as the means ± SD.The significance of the differences in mean values among the control and treated groups was evaluated following Student *t*-test using Minitab software version (16).

RESULTS

Tolerance of animals

Four days following the beginning of the treatment, animals receiving Brodifacoum seemed to have less activity, especially those treated with the highest dose compared to the control ones. It is worth mentioning that two rabbits of this groups died by the end of the experimental period. Besides, all treated individuals had a rather important alopecia's regions.

Body weight

Changes in body weight are shown infigure1. Body weight underwent a significant decrease in all treated groups as compared to the control group.

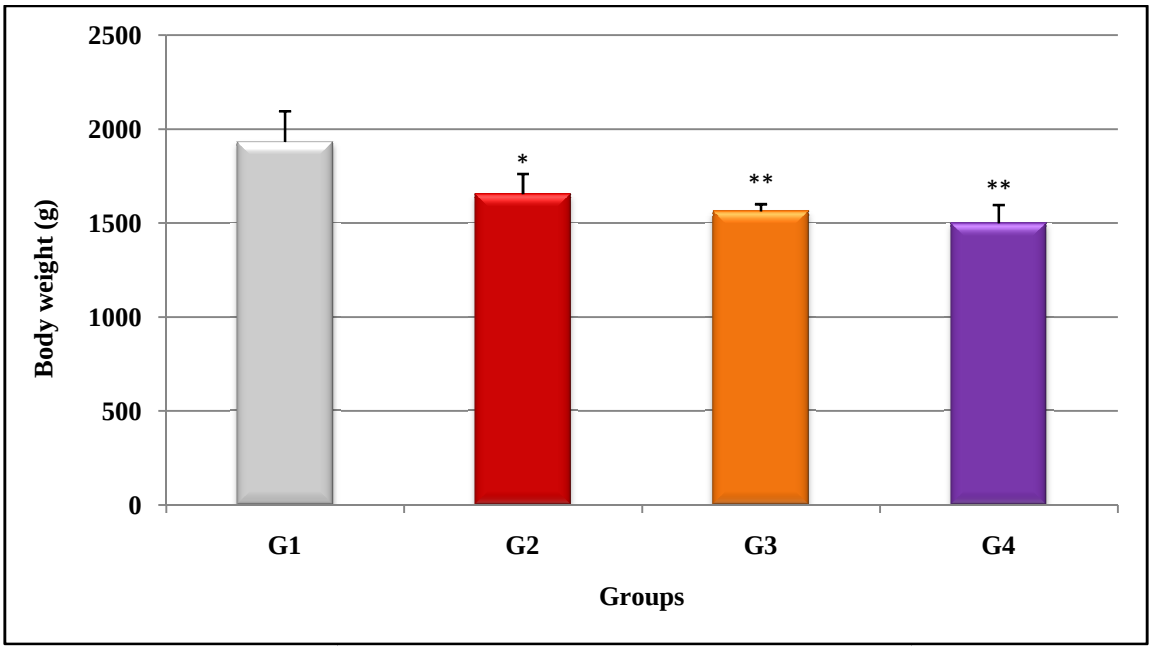

Figure 1: Variation of body weight after3 weeks of treatment in control (G1) and treated groups (G2, G3 and G4) (mean±SD, n=7)

*(P<0.05) * ;(P<0,01) ***

Organs weight

Changes in liver and kidney weights are reported in table 1. An increase in liver weight was observed in the treated animals compared to controls. In contrast, both kidney and spleen weights decreased in treated rabbits compared also to control ones.

Table 1: Variations of organs weight in control (G1) and treated groups (G2, G3 and G4) after 3 weeks of treatment

	G1 Control	G2 0.01 mg/kg	G3 0.02 mg/kg	G4 0.04 mg/kg
Liver (g)	32.07±2.91	33.50±1.53	37.04±2.07*	43.57± 3.96**
Kidney (g)	6.142±0.476	5.385±0.657	5.423±0.572	5.573±0.555
Spleen (g)	0.783±0.0887	0.893±0.174	1.444±0.340*	1.937±0.553*

*(P<0.05) *;(P<0.01) ***

Hematological parameters

Changes in hematological parameters in all groups of rabbits are summarized in figures 2, 3, 4, 5 and 6. The results reveal that administration of Brodifacoum induced a significant increase in the number of white blood cells especially in rabbits treated with 0.04mg/kg. On the other hand, a significant decrease in red blood cells, hemoglobin, hematocrit and thrombocytes concentrations was observed in treated groups compared to the control one.

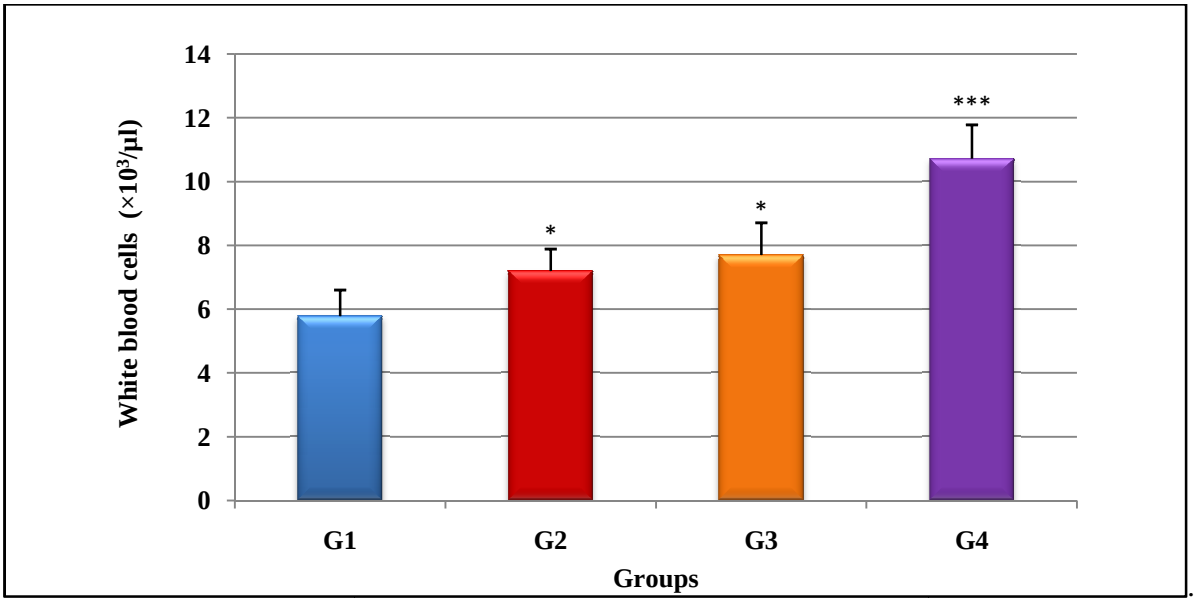

Figure 2: The measured white blood cells value after 3 weeks of treatment in control (G1) and treated groups (G2, G3 and G4)
(mean±SD, n=7)
*(P<0.05) *; (P<0.01) **; (P<0.001) ****

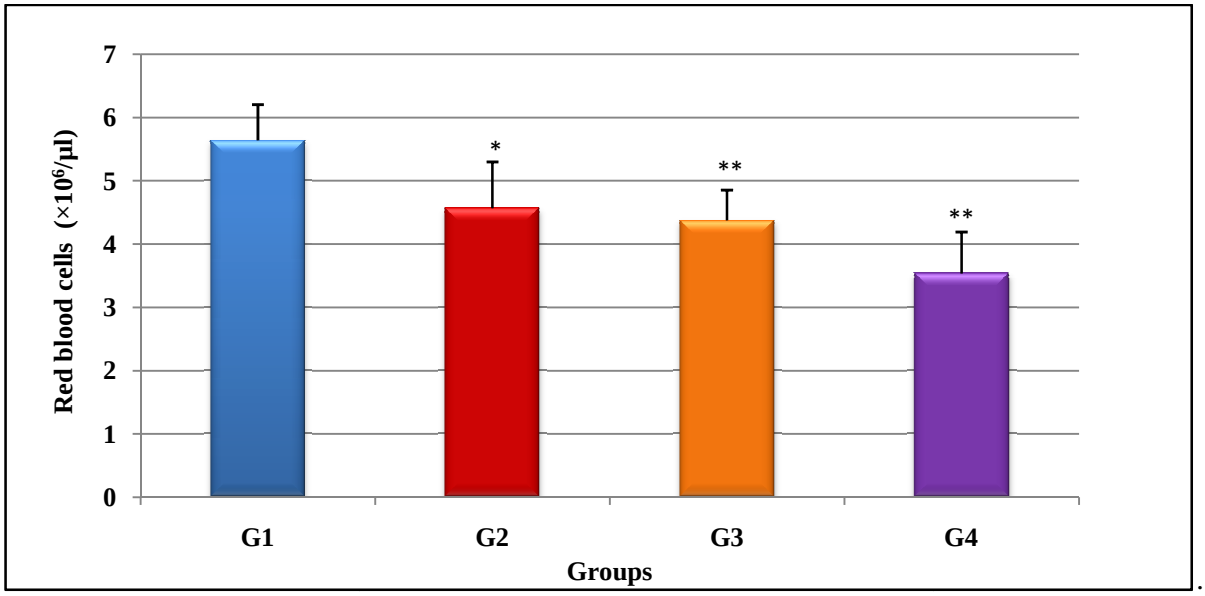

Figure 3: The measured red blood cells value after 3 weeks of treatment in control (G1) and treated groups (G2, G3 and G4)
(mean±SD, n=7)
*(P<0.05) *; (P<0.01) **; (P<0.001) ****

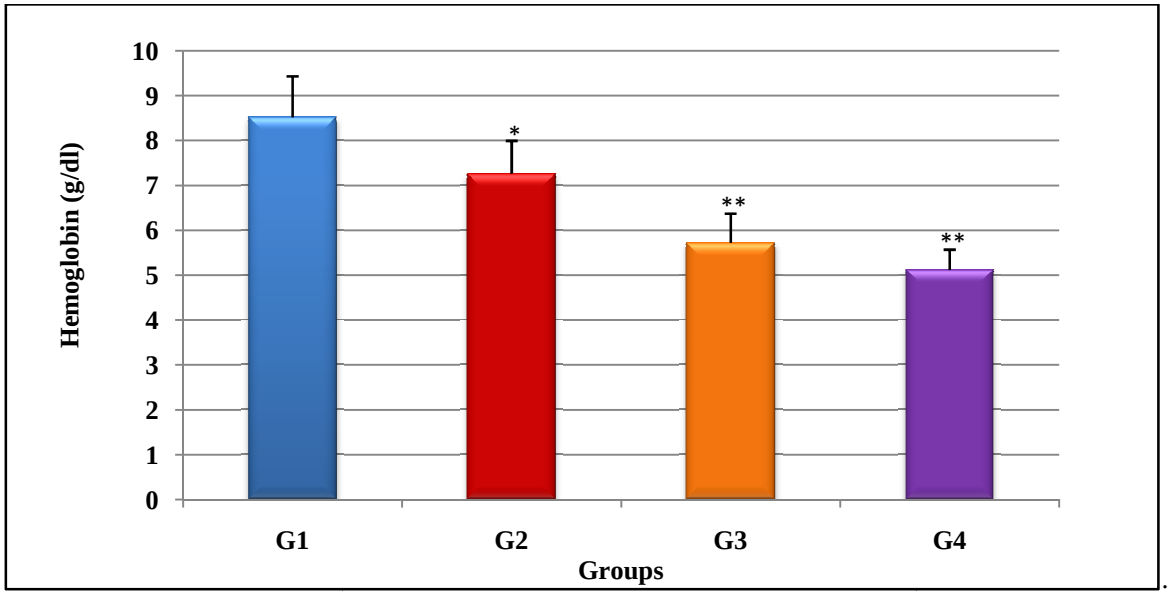

Figure 4: The measured hemoglobin value after 3 weeks of treatment in control (G1) and treated groups (G2, G3 and G4)
(mean±SD, n=7)
*(P<0.05) *; (P<0.01) ***

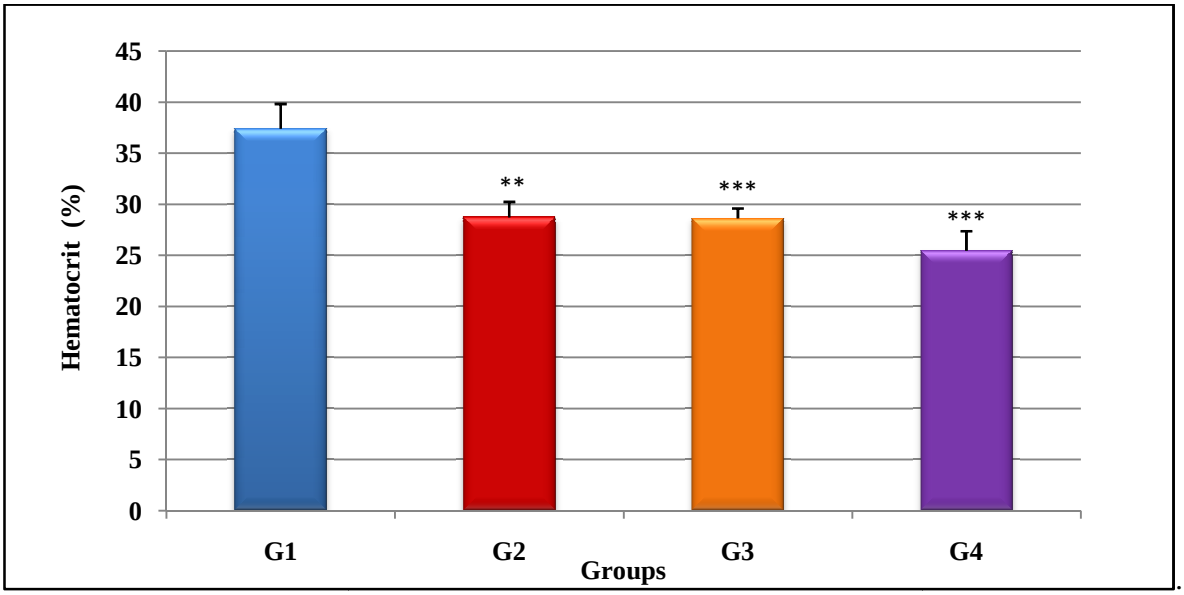

Figure 6: The measured hematocrit value after 3 weeks of treatment in control (G1) and treated groups (G2, G3 and G4)
(mean±SD, n=7)
*(P<0.05) *; (P<0.01) **; (P<0.001) ****

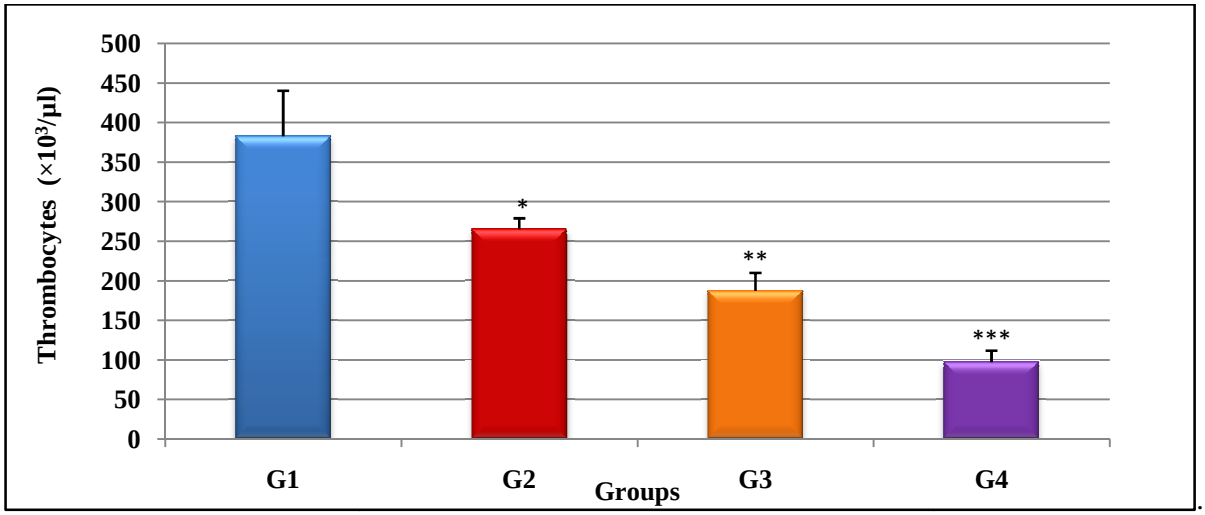

Figure 7: The measured value of thrombocytes counts after 3 weeks of treatment in control (G1) and treated groups (G2, G3 and G4)
(mean ±SD, n=7)
*(P<0.05) *; (P<0.01) **; (P<0.001) ****

Biochemical parameters

As shown in table 2, it is clear that administration of Brodifacoum in rabbits caused a very significant increase in both plasma glucose and triglycerides concentrations, compared to controls. Similarly, a significant increase in plasma cholesterol, creatinine and urea concentrations was observed in treated rabbits, compared to those of control individuals. Yet, a marked reduction in plasma uric acid concentrations was noted in treated rabbits, compared also to control ones.

Figure 7 showed a decrease in plasmatic calcium concentrations in rabbits after treatment with Brodifacoum compared to controls.

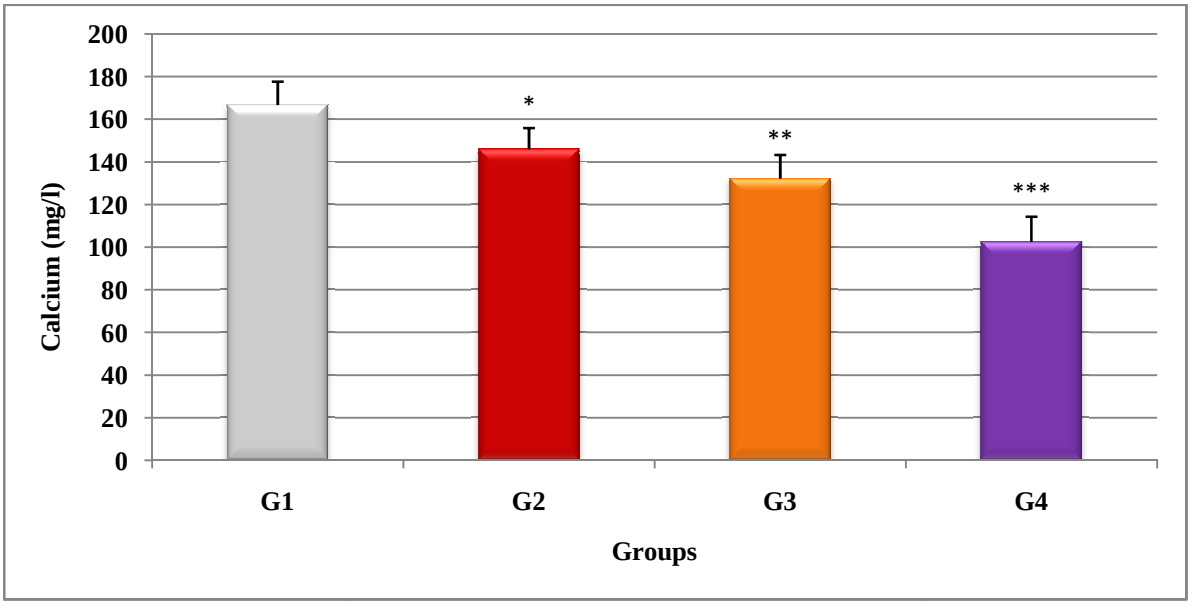

Figure 5: variation of plasmatic calcium concentrations after 3 weeks of treatment in control (G1) and treated groups (G2, G3 and G4)
(mean±SD, n=7)
*(P<0.05) *; (P<0.01) **; (P<0.001) ****

Table2: Variation of biochemical's parameters in control (G1) and treated groups (G2, G3 and G4) after 3 weeks of treatment

	G1 Control	G2 0.01mg/kg	G3 0.02mg/kg	G4 0.04mg/kg
Glucose (g/l)	1.444±0.0527	1.264±0.103*	1.146±0.104**	0.902 ±0.102***
Cholesterol (g/l)	0.58±0.0539	0.676±0.081	0.766±0.079**	0.912±0.089***
Triglycerides (g/l)	1.512±0.142	1.268±0.111*	1.106±0.0723**	0.722±0.129***
Uric Acid (mg/l)	45.84±1.06	42.80±1.96*	40.06±3.00*	38.08±2.58**
Urea (g/l)	0.284±0.049	0.376±0.034*	0.422±0.037**	0.564±0.039***
Creatinine (mg/l)	7.014±0.677	8.050±0.553*	8.262±0.690*	10.220±0.419***

($P<0.05$) * ;($P<0.01$) ** ;($P<0.001$) ***

DISCUSSION

The first observation arising from the current investigation is that administration of Brodifacoum to male rabbits induces a remarkable less activity. According to Valchev *et al.*[64], Brodifacoum cause several clinical symptoms such as non-specific-somnolence, weakness, decreased (anorexia) or lacking appetite, decreased locomotion and rapid and easy exhaustion. Our results showed a decrease in rabbit's body weight after Brodifacoum treatment in a dose depending manner. Similar observations have been reported by [65], whom demonstrated that the treatment of horses with 0.125 mg/kg of Brodifacoum revealed a decrease in body weight. On the other hand, the increase in liver weight in treated rabbits may be due to the fact that such an organ remains the main site of accumulation and storage of any exogenous substances. It is also possible that the increase in liver weight in treated rabbits is due to the slow biodegradation process of Brodifacoum in the liver as it has been reported in previous studies [59, 66]. Besides, few studies have reported that the second-generation of anticoagulants are mainly eliminated as unchanged compounds [59, 67]. The low urinary excretion precludes isolation of metabolites from the urine. The elimination of Brodifacoum is probably similar to that of warfarin: compounds resulting from the hydroxylation in the hepatocellular mixed function oxidases system are excreted in the urine [68].

The altered blood parameters in treated rabbits are in total agreement with other studies where it has been reported that the target system is the hematological system, with impairment of clotting[59].Many authors have suggested that Brodifacoum acts by inhibiting the vitamin K epoxide reductase in the vitamin K1-epoxide cycle[69].The cyclic regeneration of vitamin K1is resulting in hypoprothrombinemia. Under physiological conditions, the oxidation of vitamin K in the hepatocyte is coupled to a carboxylation step essential for activation of prothrombin factors from inactive precursors[70]. Brodifacoum produces hypoprothrombinaemia because the coupled carboxylationre action is inhibited [71].The increase in the concentrations of leucocytes following Brodifacoum administration can be explained simply by the fact that they represent the first line of defense in the organism. Moreover, an external hemorrhage was observed in treated rabbits, explaining the recorded reduction in red blood cells, hemoglobin and hematocrit.

The studies of biochemical parameters have significant value in toxicological evaluations as alterations appear quite before the clinical symptoms produced by the toxicants [72]. In this study, we found that concentration of glucose markedly decreased in treated groups. The simple explanation to this decrease could be that rats consumed less feed. The same results were obtained by [73] in house rats, after Brodifacoum administration. However, an increase in plasma glucose level was found in previous studies in rats treated with anticoagulants [46, 73]. Obtained results demonstrated a disorder in renal function revealed by an increase of urea and creatinine concentrations, and a decrease in uric acid plasma levels. The most important function of the kidney is to maintain a normal physiological condition of the body. Renal failure is mainly caused by exogenous poisons, which interferes with this function. Ingestion of such chemical substance would result in electrolyte imbalance is constantly associated with acute tubular degeneration which may finally lead to renal failure. The chemical poison present in bromadiolone decreases the excretory capacity of the kidney, by causing its damage [74]. Likewise, several authors have shown that Brodifacoum caused a kidney dysfunction revealed by a perturbation in renal parameters [75, 76].

Concerning lipid parameters, an alteration was observed in treated rabbits compared with the control ones, this probably due to Brodifacoum effect.

Finally, the decrease in the level of serum calcium in treated rabbits demonstrated the toxic effect of Brodifacoum on calcium metabolism, as it may act as a calcium substitute in second messenger metabolism. Brodifacoum reduces blood clotting factors because some of them carry gamma carboxylated residues which allow them to fix the calcium and to be bound in phospholipidic membranes [77].

Therefore, it is concluded that body weight and organs weight were significantly altered in Brodifacoum treated groups. Our results provide also that hematological and biochemical parameters were affected.

CONCLUSION

In conclusion, the present data is important because it is one of few original reports on Algeria with Brodifacoum poisoning. The results issued from this work confirm that the exposition to such substances affect many physiological parameters. The hematological and biochemical parameters are the main bioindincators showing the toxic effect of this anticoagulant rodenticide. Accordingly, care must be taken into account to avoid mammalian and human exposure to Brodifacoum. The evaluation of other parameters implicated in the detoxification processes have to check further.

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