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# BIOCHEMICAL EFFECTS OF CALCIFEROL RODENTICIDE ON ALBINO MICE

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## ABSTRACT

Median lethal dose of Claciferol (Vitamin D) against albino mice *Mus musculus* was estimated. Calciferol could be classified as a highly toxic compound to albino mice as its $LD_{50}$ values to the (immature and adult) were 47 and 60 mg/kg, respectively. Three doses representing 1/10, 1/20 and 1/30 of the $LD_{50}$ were administered orally for 2 weeks.

In treated animals the rate of body weight gain, water and food consumption were decreased clearly at 1/10 $LD_{50}$ of Calciferol than other doses. Similarly, organs weight/body weight ratio was lower than that observed in non-treated animals.

Calcium, phosphorus and cholesterol levels were affected. The tissue-specific enzymes alkaline phosphates (AP), the amount of urea, creatinine and total proteins of serum, liver and kidney were significantly increased.

**Keywords:** Calciferol, calcium, phosphorus, (AP), Cholesterol, urea, creatinine and total proteins.

## INTRODUCTION

Pest control chemicals are poisons they may present immediate danger to the user if applied improperly or without sufficient knowledge of their toxic effects.

Some are highly toxic and may cause serious illness and even death if spilled on the skin, inhaled or otherwise used carelessly.

Man has been always faced with problems of rodent which proved to be one of the most important pests all over the world, these animals belong to the largest mammalian order rodentia which contains on the average 400 genera and over 600 species, because of their primitive structure, these animals tend to be less specific than other groups of mammals and therefore more adaptable to environmental changes.

Food losses due to rodent depredations have been estimated as 33 million metric tones per year of stored grain (Moje and Metcalf 1969). At the beginning of 1980's Egypt faced a large rodent problem in agricultural area. The present study dealing with Calciferol (vitamin D) as a rodenticide other than anticoagulants vitamin D is one of the most important essential bioregulators of $Ca^{+2}$ and phosphate metabolism in higher animals. Two forms of vitamin D are presented: ergocalciferol called vitamin $D_2$ and cholecalciferol called Vitamin $D_3$ Vitamin $D_2$ is formed by the irradiation of ergosterol vitamin $D_3$ is the natural form of vitamin D in animals. Vitamin $D_2$ and $D_3$ are equally active in mammals but vitamin $D_2$ is not very active in birds (Koshy 1982).

Overdose of vitamin D is toxic in most adults after intake of more than 50.000 IU (1.24 mg) Calciferol/body (Withelm Friedrich 1988). Ingestion of an overdose of vitamin D is quickly followed by weakness,

nousea, loss of appetite, headache, abdominal pains, cramps and diarrhea (Holmes and Kummero 1983).

The aim of the present study was to investigate the effect of claciferol on calcium, phosphorous, alkaline phosphatase, cholesterol, urea, creatinine and total proteins of serum, liver and kidney.

## MATERIALS AND METHODS

Male albino mice *Mus Musculus* at immature stage (7 weeks) and adult (10 weeks) were used in this experiment. The mice were housed in an air conditioned laboratory and fed a standard commercial diet and water ad-labitum. The animal were starved for 12 hours before dosing master mix of Calciferol rodenticide (3.75% a.i.) obtained from KZ company. Serial dilutions of Calciferol were prepared and administered orally. Each tested concentration included 10 animals, and was replicated two times. A control check test was run. Mortality counts were done 24 hours after treatment and mortality curves were drawn on probit papers according to Finney (1952). The median lethal dose ($LD_{50}$) was estimated and several groups of adult albino mice were treated with 1/10, 1/20 and 1/30 $LD_{50}$ of Calciferol. Animals were weighed every day and their daily food intake was assessed. Treated mice and the control group were sacrificed at two weeks post-treatment. Blood was collected. Liver, brain, kidney and spleen were dissected out and weighed. Diet and water were supplied ad labitum.

Serum, liver and kidney were subjected to several biochemical determination, total protein was determined according to the method of Biuret, Captain *et al* (1946). Calcium was determined to the method of Tietz (1970).

Inorganic phosphour was determined according to the method of Goldenberg *et al* (1966). Alkaline phosphatase activity was determined by the method of Kind and king (1954). Cholesterol was determined according to the method of Watson (1960). Creatinine was assayed by the method of Henry (1974), while urea was assayed by the method of Patton and Crouch (1977).

## RESULTS AND DISCUSSION

In Table (1), the results indicate that immature stage of mice was more sensitive than adult. This may be due to high activity of growing animals through this period of development. This finding is not surprising because it is well known that pesticides are more toxic in the immature stage than in adult (Meehan, 1984).

Data in Tables (2,3 and 4) show the effect of Calciferol on the body weight of adult mice. It was observed that the body weight, food and water consumption were decreased in animal treated with 1/10 $LD_{50}$ Calciferol more than those treated by other doses, this decrease in body weight was reflected in the decrease of organs weight/body weight ratio. The present results are in agreement with those obtained by Gaines (1969).

**Table (1): Acute toxicity of oral (PO) Calciferol to different ages of adult albino mice.**

| Rodenticide | LD50 mg/kg (PO) Age of Mice | |
|---|---|---|
| **Stage of Mice** | **Immature Stage** | **Adult** |
| Claciferol | 47 | 60 |
| VS/immature | 1.0 | 1.276 |

**Table (2): Body weight gain of male albino mice exposed to 1/10, 1/20 and 1/30 $LD_{50}$ Claciferol for two weeks.**

| Treatment | Initial body weight | Final body weight | Body weight gain | Daily body weight gain | |
|---|---|---|---|---|---|
| | (g) | (g) | (g) | (g) | % |
| Control | 22.5 ± 0.90 | 23.53 ± 0.94 | 1.03 ± 0.04 | 0.07 ± 0.007 | 100 |
| 1/10 $LD_{50}$ | 21.3 ± 0.85 | 21.92 ± 0.88 | 0.62 ±0.02 | 0.04 ± 0.004 | 57 |
| 1/20 $LD_{50}$ | 21.8 ± 0.87 | 22.62 ± 0.90 | 0.82 ± 0.03 | 0.05 ± 0.005 | 71.4 |
| 1/30 $LD_{50}$ | 22.2 ± 0.89 | 23.13 ± 0.92 | 0.93 ± 0.03 | 0.066± 0.006 | 94 |

***% at control** **Mean values ± SE**

**Table (3): Food and water consumption of male albino mice exposed to1/10, 1/20 and 1/30 $LD_{50}$ Claciferol for two weeks.**

| Treatment | Food intake | Water intake | Daily food intake | | Daily water intake | | Daily body weight gain | |
|---|---|---|---|---|---|---|---|---|
| | (g) | (ml) | (g) | (%*) | (ml) | (%*) | (g) | (%*) |
| **Control** | 49.0 ± 4.3 | 70.0 ± 6.3 | 3.5 ± 0.34 | 100 | 5.0 ± 0.47 | 100 | 0.07 ± 0.007 | 100 |
| **1/10 $LD_{50}$** | 41.0 ± 3.6 | 63.0 ± 5.7 | 3.1 ± 0.30 | 88 | 4.5 ± 0.42 | 90 | 0.04 ± 0.004 | 57 |
| **1/20 $LD_{50}$** | 45.0 ± 3.9 | 66.0 ± 5.9 | 3.2 ± 0.31 | 91 | 4.7 ± 0.44 | 94 | 0.05 ± 0.005 | 71.4 |
| **1/30 $LD_{50}$** | 47.0 ± 4.1 | 68.0 ± 6.1 | 3.4 ± 0.33 | 97 | 4.8 ± 0.45 | 96 | 0.066 ± 0.006 | 94 |

***% VS control** **Mean values ± SE**

**Table (4): Organs weight/body weight ratio of male albino mice exposed to 1/10, 1/20 and 1/30 $LD_{50}$ Claciferol for two weeks.**

| Treatment | Liver weight | | Liver w./body w.ratio | | Kidney weight | | Kidney w./body w. ratio | |
|---|---|---|---|---|---|---|---|---|
| | (g) | (%*) | Ratio | (%*) | (g) | (%*) | Ratio | (%*) |
| **Control** | 1.28 ± 0.12 | 100 | 0.057 ± 0.005 | 100 | 0.29 ± 0.024 | 100 | 0.012 ± 0.001 | 100 |
| **1/10 $LD_{50}$** | 1.05 ± 0.098 | 82 | 0.049 ± 0.004 | 86 | 0.22 ± 0.018 | 76 | 0.010 ± 0.001 | 83 |
| **1/20 $LD_{50}$** | 1.15 ± 0.11 | 89 | 0.052 ± 0.005 | 91 | 0.25 ± 0.020 | 86 | 0.011 ± 0.001 | 92 |
| **1/30 $LD_{50}$** | 1.20 ± 0.11 | 93 | 0.054 ± 0.005 | 94 | 0.27 ± 0.022 | 93 | 0.012 ± 0.001 | 100 |

***% VS control** **Mean values ± SE**

Results recorded in Tables (5 and 6) illustrated the effect of Calciferol effects on the level of calcium in serum, liver and kidney of treated animals. It is apparent that calcium was significantly increased. In contrary phosphorus level was significantly decreased. These findings are in agreement with those obtained by Richard and Follis (1955) and Corocker *et*

*al* (1985). Similarly alkaline phosphatase activity was significantly increased in animals subjected to the three tested doses (1/10, 1/20, 1/30 of $LD_{50}$).

**Table (5): Calcium $Ca^{+2}$ level in serum, liver and kidney of male albino mice treated with different doses of Calciferol for two weeks.**

| Treatment | 14 Days Post-treatment | | |
|---|---|---|---|
| | Serum | Liver | Kidney |
| Control | 12.4 ± 0.73 | 0.17 ± 0.012 | 0.14 ± 0.007 |
| 1/10 $LD_{50}$ | 14.0 ± 0.82 | 0.20 ± 0.014 | 0.17 ± 0.008 |
| 1/20 $LD_{50}$ | 13.7 ± 0.80 | 0.19 ± 0.013 | 0.15 ± 0.007 |
| 1/30 $LD_{50}$ | 13.9 ± 0.82 | 0.18 ± 0.013 | 0.15 ± 0.007 |

**Each value represented mean (mg/L) ± SE of 5 mice**

**Table (6): Phosphorus level in serum, liver and kidney of male albino mice treated with different doses of Calciferol for two weeks.**

| Treatment | 14 Days Post-treatment | | |
|---|---|---|---|
| | Serum | Liver | Kidney |
| Control | 11.95 ± 0.40 | 0.08 ± 0.008 | 0.05 ± 0.01 |
| 1/10 $LD_{50}$ | 6.57 ± 0.22 | 0.042 ± 0.004 | 0.041 ± 004 |
| 1/20 $LD_{50}$ | 8.32 ± 0.28 | 0.065 ± 0.006 | 0.039 ± 003 |
| 1/30 $LD_{50}$ | 11.16 ± 0.37 | 0.074 ± 0.007 | 0.042 ± 004 |

**Each value represented mean (mg/L) ± SE of 5 mice**

Significant increases in activity of alkaline phosphatase (AP) and cholesterol in serum, liver and kidney were observed (Tables 7 and 8). Similar results were obtained by Levi *et al* (1987) and Guven *et al* (1990) who found that serum alkaline phospatase (AP) was increased when toxic doses of cholecalciferol (Vit. $D_3$) were injected subcutaneously into rats for 5 weeks.

Data in Table (9) show the effect of Calciferol on the urea and creatinine. It was observed that urea and creatinine in the high dose of 1/10 $LD_{50}$ was increased in kidney and liver more than other doses.

**Table (7): Alkaline phosphotase activities (unit/g) in liver and kidney of male albino mice treated with different doses of Calciferol for two weeks.**

| Treatment | 14 Days Post-treatment | |
|---|---|---|
| | Liver | Kidney |
| Control | 9.4 ± 0.10 | 8.7 ± 0.6 |
| 1/10 $LD_{50}$ | 14.2 ± 0.15 | 11.5 ± 0.79 |
| 1/20 $LD_{50}$ | 13.5 ± 0.14 | 10.4 ± 0.72 |
| 1/30 $LD_{50}$ | 11.6 ± 0.12 | 9.2 ± 0.63 |

**Mean values ± SE * unit/ml.**

**Table (8): Cholesterol in serum of male albino mice treated with different doses of Calciferol for two weeks.**

| Treatment | 14 Days Post-treatment |
|---|---|

| | |
|---|---|
| Control | 0.28 ± 0.006 |
| 1/10 $LD_{50}$ | 0.33 ± 0.007 |
| 1/20 $LD_{50}$ | 0.30 ± 0.006 |
| 1/30 $LD_{50}$ | 0.29 ± 0.006 |

**Each value represented mean (g/L) ± SE of 5 mice.**

**Table (9): Urea and Creatinine level in liver and kidney of male albino mice treated with different doses of Calciferol for two weeks.**

| Treatment | 14 Days Post-treatment | | | |
|---|---|---|---|---|
| | Liver | | Kidney | |
| | Urea | Creatinine | Urea | Creatinine |
| Control | 5.2 ± 0.11 | 0.78 ± 0.016 | 6.4 ± 0.2 | 0.96 ± 0.020 |
| 1/10 $LD_{50}$ | 5.7 ± 0.12 | 0.85 ± 0.017 | 7.2 ± 0.23 | 1.08 ± 0.022 |
| 1/20 $LD_{50}$ | 5.4 ± 0.11 | 0.82 ±0.017 | 6.8 ± 0.21 | 1.02 ± 0.021 |
| 1/30 $LD_{50}$ | 5.0 ± 0.11 | 0.80 ± 0.016 | 6.6 ± 0.21 | 0.99 ± 0.020 |

**Each value represented mean (g/L) ± SE of 5 mice.**

The present results are in agreement with those obtained by Varley (1976) who indicated that plasma urea and creatinine increases in renal diseases gave prognostic significant those of other nitrogen substances. Also, Honda *et al.* (1992) and El-Halwagy (1995) noticed an increased in serum urea and creatinine of female subjects exposed to cadmium and certain rodenticide.

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## التأثيرات البيوكيماوية لمبيد القوارض الكالسيفيرول على فئران التجارب

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أجريت دراسة السُمية تحت الحادة لمبيد القوارض الكالسيفيرول على فئران التجارب بإستخدام ثلاث جرعات للمركب المختبر وهى 10/1 ، 20/1، 30/1 من الجُرعة السامة النصفية بطريق الفم ومتابعة مجاميع الحيوانات لمركب المختبر لمدة أربعة عشر يوماً من المُعاملة.

وأوضحت النتائج أن هناك علاقة بين تأثير الجرعة على معدلات النمو ومستوى عنصر الكالسيوم والفوسفور و الكوليسترول والنشاط الإنزيمى للفوسفاتيز القلوى واليوريا والكرياتنين وذلك بالسيرم والكبد والكلى وأصبحت واضحة على مستوى الجرعة العالية وأنخفضت بوضوح على مستوى الجرعة المنخفضة لمبيد الكالسيفيرول.