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**SERUM TRIIODOTHYRONINE, THYROXINE,
AND THYROID STIMULATING HORMONE
CONCENTRATIONS IN DONKEYS FOLLOWING
ADMINISTRATION OF DORAMECTIN
(EXPERIMENTAL STUDY)
(With 3 Tables)**

By

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**تركيز الثايرونين ثلاثي اليود، الثايروكسين والهرمون المنشط للدرقية
في الحمير عقب حقن عقار الدورامكتين (دراسة تجريبية)**

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تم إجراء دراسة لتقويم التسمم دون الحاد للدورامكتين المحقون في الحيوانات السليمة إكلينيكيًا. التجربة شملت أربع مجموعات من الحيوانات، كل مجموعة ضمت خمسة حمير. ثلاث مجموعات تم حقنها بعقار الدورامكتين في العضل بجرعات مقدارها: ١٠٠، ٢٠٠ و ٣٠٠ ميكروجرام لكل كيلوجرام وزن حي لمدة سبعة أيام متتالية، وتركت مجموعة ضابطة. تم تجميع عينات الدم يوميًا في الأسبوع الأول ثم كل أسبوع لمدة أربعة أسابيع إضافية. النتائج التي تم الحصول عليها من المجموعات المعالجة تمت مقارنتها بمجموعة التحكم. تم استقصاء تأثير عقار الدورامكتين علي هرمونات الثايروكسين، الثايرونين ثلاثي اليود والهرمون المنشط للدرقية. التآرجح الذي حدث في مستوي الهرمونات، في المجموعات التي تم علاجها، لم يظهر فروقاً معنوية بينه وبين مستوي الهرمونات في مجموعة التحكم. وعليه يمكن القول بأن عقار الدورامكتين عند حقنه بجرعات مقدارها ١٠٠، ٢٠٠ و ٣٠٠ ميكروجرام لكل كيلوجرام وزن حي لمدة أسبوع كامل ليس له أي سمية أو آثار جانبية سيئة علي فصيلة الحمير.

SUMMARY

The objectives of this experiment were to determine serum concentrations of Triiodothyronine (T₃), Thyroxine (T₄), and Thyroid

Stimulating Hormone (TSH) following administration of the anthelmintic Doramectin in healthy donkeys. This was done to investigate the sub acute effect of doramectin injectable formulation in donkeys. Serum T₃, T₄ and TSH concentrations in serum samples obtained before and after doramectin administration for continuous 7 days, were determined using RIA technique. Baseline values ranged from 0.32 to 1.63 ng of T₃/ ml, 3.13 to 22.61 ng of T₄/ ml, and 0.34 – 1.01 mIU/L of TSH. There are no significant (P>0.05) differences between the treatment groups and the control for the concentration of the three hormones under investigation, but there is significant increase in the level of hormones when compared with the pre treatment level in all groups.

Key words: *Doramectin, donkeys, Triiodothyronine, Thyroxine, Thyroid Stimulating hormone.*

INTRODUCTION

Doramectin (DRM) is an avermectin compound obtained by mutational biosynthesis, by feeding cyclohexane carboxylic acid to a genetically – engineered strain of *S. avermitilis* and, as a result, has a cyclohexyl group at the C₂₅ position (Goudie *et al.*, 1993). Doramectin and Ivermectin are endoectocide compounds with exceptional potency. They have broad nematode and arthropod spectra.

Normal thyroid hormone concentrations reported for horses have a wide range of values (Kallfelz and Erali, 1973; Reap *et al.*, 1978; and Thomas and Adams, 1978). Several intrinsic and extrinsic factors influence the concentrations, including age (Irvine and Evans, 1975), season (Katovich *et al.*, 1974), stage of training (Takagi *et al.*, 1974) and pregnancy (De Martin, 1977). Using of medicines also had an effect for example phenylbutazone has been shown to decrease thyroid hormone concentrations (Morris and Garcia, 1983, Sojka *et al.*, 1993).

The aim of this study was to investigate the possible toxic effects, if any, of administration of Doramectin Injectable formulation for seven continuous days intramuscularly on T₃, T₄ and TSH hormones.

MATERIALS and METHODS

Design of the experiment: Four groups of animals were used in this study. Each group comprised of five animals (three male and two female

donkeys). They were 4-10 years old. The animals in the treatment groups (DRMTOX1, DRMTOX2 and DRMTOX3) received a daily intramuscular injection of Doramectin (Dectomax injection 1.0% w/v sterile solution of Doramectin, Pfizer, France) for a whole week, at three different dose levels viz: 100, 200 and 300 µg/kg body weight, respectively. The fourth group remained untreated as control group. Then blood samples were collected for hormonal assays, after each injection animals were monitored for two hours for any untoward effects.

Collection of blood: Blood samples were collected before the administration of the drug (day zero) and then daily for the first week after the administration of the drug and then every week for three additional weeks. Whenever blood samples were taken they were allowed to clot, the clotted blood samples were centrifuged and sera were separated and stored at -20 °C until analyzed.

Hormones detection: Thyroxine (T₄) and triiodothyronine (T₃) were measured in donkey's serum using RIA Kit (Beijing Atom High-tech. Co. LTD). Whereas thyroid stimulating hormone (TSH) was measured using the IRMA technique. The principal method of this immunoradiometric assay (IRMA) kit utilizes two site sandwich immuno-radiometric assay for the measurement of TSH in serum. This involves the reaction of TSH present in serum with monoclonal and polyclonal antibody. The monoclonal antibody is labeled with I¹²⁵ as tracer (I¹²⁵-McAb) and the polyclonal antibody is coupled to magnetic iron oxide particle (PcAb {M}). The formed complex was separated using a magnetic separator.

The values were expressed in ng/ml for T₃ and T₄ whereas for TSH in mIU/L. However, both methods used (RIA and IRMA) are able to detect the thyroid hormones with sensitivity of 0.08 ng/ml, 0.13 ng/ml, and 0.24 mIU/L for T₃, T₄ and TSH respectively.

Statistical methods: SPSS 11.5 for Windows computer package was utilized in assessing significant differences, if any. Analysis of variance (ANOVA) was used to compare between means.

RESULTS

Table 1: Changes in triiodothyronine (T₃) level (ng/ml) following administration of doramectin at three different dose rate.

Days	Control	DRMT1	DRMT2	DRMT3
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	0.88 ± 0.40*	0.79 ± 0.24*	1.21 ± 0.37*	0.85 ± 0.17*
1	1.00 ± 0.33	1.01 ± 0.80	1.84 ± 0.53	1.37 ± 0.87
2	0.88 ± 0.55	0.87 ± 0.44	1.03 ± 0.47	0.74 ± 0.48
3	0.77 ± 0.37	0.92 ± 0.40	1.21 ± 0.22	0.86 ± 0.27
4	1.16 ± 0.31	1.00 ± 0.70	0.74 ± 0.25	0.72 ± 0.61
5	2.25 ± 0.49*	1.96 ± 0.36*	2.21 ± 0.42*	2.31 ± 1.01*
6	2.01 ± 0.25*	2.12 ± 0.11*	1.82 ± 0.24	2.27 ± 0.99*
7	1.94 ± 0.48*	1.64 ± 0.34*	1.70 ± 0.24	1.86 ± 0.41*
14	2.13 ± 0.93*	2.23 ± 0.41*	2.77 ± 1.12*	2.23 ± 0.35*
21	2.38 ± 0.76*	1.88 ± 0.79*	2.55 ± 0.57*	2.04 ± 0.50*
28	2.57 ± 0.97*	1.70 ± 0.43*	1.87 ± 0.34	2.03 ± 0.45*

*Significant difference (P<0.05) in comparison with day zero.

Table 2: Changes in thyroxine (T₄) level (ng/ml) following administration of doramectin at three different dose rate.

Days	Control	DRMT1	DRMT2	DRMT3
	Mean ±SD	Mean ± SD	Mean ± SD	Mean ± SD
0	10.47 ± 8.56*	10.23 ± 5.49*	12.12 ± 7.42*	11.72 ± 3.41*
1	14.60 ± 10.86	7.86 ± 6.17	13.16 ± 9.48	9.66 ± 11.81
2	4.21 ± 4.40	5.81 ± 3.89	10.00 ± 5.28	7.01 ± 4.34
3	8.35 ± 7.48	5.42 ± 2.68	6.54 ± 1.14	3.60 ± 2.74
4	11.61 ± 7.14	4.93 ± 1.25	11.08 ± 8.67	7.58 ± 3.77
5	28.94 ± 5.87*	36.53 ± 15.88*	30.11 ± 10.08	31.03 ± 13.91*
6	26.55 ± 15.74*	21.55 ± 5.64*	36.39 ± 12.85*	23.21 ± 5.26*
7	18.46 ± 6.87	21.76 ± 5.33*	54.06 ± 34.48*	30.73 ± 12.38*
14	16.47 ± 9.07	17.44 ± 4.38	14.70 ± 5.29	14.98 ± 4.02
21	19.46 ± 3.64	21.98 ± 9.26*	24.10 ± 9.50	22.33 ± 7.70
28	32.86 ± 19.13*	24.40 ± 7.69*	24.39 ± 13.29	35.49 ± 4.70*

* Significant difference (P<0.05) in comparison with day zero.

Table 3: Changes in thyroid stimulating hormone (TSH) level (mIU/L) following administration of doramectin at three different dose rate.

Days	Control	DRMT1	DRMT2	DRMT3
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
0	0.70 \pm 0.30	0.55 \pm 0.40	0.59 \pm 0.47	0.58 \pm 0.41
1	0.72 \pm 0.53	0.93 \pm 0.53	0.77 \pm 0.14	0.72 \pm 0.15
2	0.71 \pm 0.49	0.64 \pm 0.49	0.90 \pm 0.21	0.47 \pm 0.50
3	0.95 \pm 0.15	0.55 \pm 0.42	1.05 \pm 0.34	0.55 \pm 0.37
4	0.89 \pm 0.33	0.57 \pm 0.25	0.58 \pm 0.50	0.68 \pm 0.07
5	1.05 \pm 0.24	0.39 \pm 0.28	0.78 \pm 0.55	0.48 \pm 0.37
6	0.89 \pm 0.79	0.75 \pm 0.34	0.97 \pm 0.29	0.69 \pm 0.19
7	0.57 \pm 0.46	0.74 \pm 0.55	0.96 \pm 0.19	0.67 \pm 0.51
14	0.87 \pm 0.13	0.87 \pm 0.36	1.03 \pm 0.12	0.72 \pm 0.57
21	1.24 \pm 0.44	1.01 \pm 0.10	0.91 \pm 0.46	0.87 \pm 0.27
28	0.93 \pm 0.22	1.36 \pm 0.79	0.93 \pm 0.55	1.07 \pm 0.52

* Significant difference ($P < 0.05$) in comparison with day zero.

DISCUSSION

In this study the effect of Doramectin injection on T_3 , T_4 and TSH profile was investigated, as shown in Table (1). There is no significant ($P > 0.05$) difference in triiodothyronine (T_3) level between the treatment groups and the control group. The level of T_3 showed simultaneous increase in the control and treated groups when compared with pretreatment level from day 5 post treatment to the end of the experiment as shown in Table (1).

From Table (2), it is to be seen that thyroxine level (T_4) did not show any significant differences between the treatment groups and the control. The level of the hormone showed simultaneous increase in all groups from day 5 post treatment and onwards when compared with the pretreatment level as shown in Table (2).

From Table (3), we could observe that thyroid stimulating hormone (TSH) level did not show any significant difference between the treatment groups, and when compared with the pretreatment level within the treatment groups as shown in Table (3).

The avermectins mechanism of toxicity in mammals is unknown but gamma amino butyric acid (GABA) is a mammalian nervous system neurotransmitter and effect on GABA may be relevant to their safety in

mammals (Lankas and Gordon, 1989). In contrast to lower organisms, GABA is found only in the brain and spinal cord of mammals, with no detectable levels in the peripheral nervous system (Cooper, 1982). However, its concentration varies in different regions, with the greatest concentrations found in the basal ganglia, hippocampus, cerebellum, and hypothalamus in the brain and in the *substantia gelatinosa* of the dorsal horn of the spinal cord (Steffey, 2001).

In this study the effect of treatment on thyroid hormones revealed non significant difference ($P>0.05$) between the treatment groups and the control which indicates that Doramectin Injectable at these dose levels did not have any effect on thyroid hormones. The increase in thyroid hormones concentration in the treated groups occurred at the same time in the control animals.

CONCLUSION

We conclude that Doramectin Injectable formulation at the dose regimen used here had no effect on thyroid stimulating hormone and thyroid hormones.

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