

# Effect of Carmoisine Orally Administered on Thyroid Hormones and Thyroid Stimulating Hormone of Albino Rats

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**Abstract:** The effect of carmoisine orally administered on thyroid hormones and thyroid stimulating hormone on albino rats were evaluated. Thirty rats (30) weighing approximately 0.14kg were used for the experiment. The rats were divided into five groups namely 1, 2, 3, 4 and 5. Each group consist of six (6) rats. When the  $T_3$ ,  $T_4$  and TSH were analysed statistically, results obtained showed that Group 1(control: 0.0g/kg) had  $0.2200 \pm 0.0609$ ;  $2.185 \pm 0.4687$  and  $0.7933 \pm 0.541$  for TSH,  $T_4$  and  $T_3$  respectively. Group 2 (0.07g/kg) had  $0.1887 \pm 0.0723$ ;  $2.847 \pm 0.7020$  and  $1.332 \pm 0.4913$  for TSH,  $T_4$  and  $T_3$  respectively. Group 3 (0.11g/kg) had  $0.3767 \pm 0.3051$ ,  $2.438 \pm 0.2255$  and  $1.375 \pm 0.5023$  for TSH,  $T_4$  and  $T_3$  respectively. Meanwhile, Group 4 (0.14g/kg) had  $0.1017 \pm 0.0279$ ,  $3.012 \pm 0.4185$  and  $1.087 \pm 0.4628$  for TSH,  $T_4$  and  $T_3$  respectively. Finally, Group 5 had  $0.0933 \pm 0.0378$ ,  $3.4370 \pm 0.5896$  and  $1.688 \pm 0.6748$  for TSH,  $T_4$  and  $T_3$  respectively. The comparison of Group 1 and Group 2 (table 4.1) and group 1 and Group 3 showed no significant differences in TSH,  $T_4$  and  $T_3$  ( $p < 0.05$ ). The comparison between group 1 and group 4 (0.14g/kg bodyweight) was significant for TSH and  $T_4$  ( $p < 0.05$ ) but was not significant for  $T_3$ . Finally, the comparison between group 1 and group 5 (0.18g/kg bodyweight) showed a significant decrease in TSH and a significant increase in  $T_4$  and  $T_3$ . The study would showed that high doses of carmoisine up to 0.14g/kg bodyweight and above induced hyperthyroidism and reduced TSH plasma levels in albino rats. The study suggested that oral administration of carmoisine dyes induced a dose-dependent increase in the plasma levels of  $T_3$  and  $T_4$  as well as reduced level of TSH of albino rats.

**Keywords:** Thyroxine ( $T_4$ ), Triiodothyronine ( $T_3$ ), Thyroid stimulating hormone (TSH), Carmoisine

## 1. Introduction

The thyroid gland as part of the endocrine system, produce stores and release hormones into the blood stream [1][2]. The thyroid gland produces two main hormones; Triiodothyronine ( $T_3$ ) and Thyroxine ( $T_4$ ) [1][3].  $T_3$  and  $T_4$  thyroid hormones synthesis is controlled by through the pituitary and hypothalamus producing thyroid stimulating hormones (TSH) and thyroid releasing hormone (TRH) respectively [1]. They are formed by addition of iodine to tyrosine amino acid [1][2][3]. The most synthesized hormone in thyroid gland is  $T_4$  while the most efficient hormone is  $T_3$  and they are stored in the colloid or cells of the follicle bound to thyroglobin [1][2]. The thyroid hormones has effect on the growth and development, regulate calorogenesis and metabolic rate throughout life [3][4]. At the molecular level, thyroid hormones increases oxygen consumption, enhance mitochondrial metabolism, increase sensitivity to catecholamines which stimulates increased heart rate and myocardial contractility, stimulate protein synthesis, increase synthesis and degradation of cholesterol and triglycerides, increase vitamin requirement and regulate calcium and phosphorus metabolism [1][3]. Several factors have been reported to influence normal thyroid functions such as drugs, chemicals, etc. However, in this study the effect carmoisine food dye on the thyroid hormones and TSH of albino rats will be evaluated.

Carmoisine is a synthetic water soluble dye that appears as red or maroon colour. It is applied in food, drugs and cosmetics industries [5][6]. They are used in food industries for many reasons such as to prevent colour loss due to storage or processing of foods, improve colour variations and enhancement of food appearance to satisfy consumers

demand and expectations. However, side effect of carmoisine have been investigated in animals and have been reported that carmoisine induces deleterious effect on organs [5][6][7][8][10]. It was also reported that carmoisine does not directly act on the thyroid gland in either species [11]. Serum TSH was increased at the highest dose and there was a significant dose-related increase in total serum iodide in all groups. It was explained that the increase in TSH was due to the effect of elevated serum iodide rather than a direct effect on the thyroid [11].

## 2. Materials and Methods

### 2.1. Materials

Materials used include lithium heparin bottles, centrifuge, stat fax 2000 micro-plate reader, refrigerator and automated pipette.  $T_3$ ,  $T_4$  and TSH reagents purchased from Bio-Check, California, USA were used for the ELISA analysis of these parameters.

### 2.2. Animals

Sixty albino rats were purchase from University of PortHarcourt animal farm, transported in a well-ventilated plastic cage and acclimatized for two weeks under normal condition (alternate day and nights) in the animal house of Department Of Medical Laboratory Science, Rivers State University of Science And Technology, PortHarcourt. The animals were fed with marsh chicken feeds and water daily.

### 2.3. Administration

A total of 30 albino rats weighing approximately 0.14kg were used in this study. The rats were divided into five (5) groups namely; 1, 2, 3, 4 and 5 with each group consisting of 6 rats. They were treated orally using gavage method with a daily intake of 1.0ml of 0.0% (0.0g/kg), 1.0% (0.07g/kg), 1.5% (0.11g/kg), 2.0% (0.14g/kg) and 2.5% (0.18g/kg) respectively. The duration of the treatment lasted for 4 weeks.

### 2.4. Collection of Specimen

Cardiac puncture was performed aseptically to collect 5ml of fasting specimen into a lithium heparin bottles with minimum stasis and was well mixed. The whole blood was spun at 4000rpm for 5 minutes. The plasma was collected into another plain bottle for analysis of triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>) and thyroid stimulating hormones (TSH).

### 2.8. Statistical Analysis

Data obtained were statistically analysed using graph pad prism 5.1. Statistical tools used were mean, standard deviation and student statistical t-test.

## 3. Result

When the specimen were analysed statistically, results obtained showed that Group 1(control: 0.0g/kg) had  $0.2200 \pm 0.0609$ ;  $2.185 \pm 0.4687$  and  $0.7933 \pm 0.541$  for TSH, T<sub>4</sub> and T<sub>3</sub> respectively. Group 2 (0.07g/kg) had  $0.1887 \pm 0.0723$ ;  $2.847 \pm 0.7020$  and  $1.3332 \pm 0.4913$  for TSH, T<sub>4</sub> and T<sub>3</sub> respectively. Group 3 (0.11g/kg) had  $0.3767 \pm 0.3051$ ,  $2.438 \pm 0.2255$  and  $1.375 \pm 0.5023$  for TSH, T<sub>4</sub> and T<sub>3</sub> respectively. Meanwhile, Group 4 (0.14g/kg) had  $0.1017 \pm 0.0279$ ,  $3.012 \pm 0.4185$  and  $1.087 \pm 0.4628$  for TSH, T<sub>4</sub> and T<sub>3</sub> respectively. Finally, Group 5 had  $0.0933 \pm 0.0378$ ,  $3.4370 \pm 0.5896$  and  $1.688 \pm 0.6748$  for TSH, T<sub>4</sub> and T<sub>3</sub> respectively. The comparison of Group 1 and Group 2 (table 3.1) and group 1 and Group 3 (table 3.2) showed no significant differences in TSH, T<sub>4</sub> and T<sub>3</sub> ( $p < 0.05$ ). The comparison between group 1 and group 4 (0.14g/kg bodyweight) (table 3.3) was significant for TSH and T<sub>4</sub> ( $p < 0.05$ ) but was not significant for T<sub>3</sub>. Finally, the comparison between group 1 and group 5 (0.18g/kg bodyweight) showed a significant decrease in TSH and a significant increase in T<sub>4</sub> and T<sub>3</sub> (table 3.4).

**Table 3.1:** Comparison of carmoisine treated rats Group 1(control) and group 2

Parameter	TSH (mU/L)	T <sub>4</sub> (µg/dl)	T <sub>3</sub> (ng/dl)
Group 1 (0.0g/kg)	$0.2200 \pm 0.0609$	$2.185 \pm 0.4687$	$0.7933 \pm 0.541$
Group 2 (0.07g/kg)	$0.1887 \pm 0.0723$	$2.847 \pm 0.7020$	$1.3332 \pm 0.4913$
p- value	0.1735	0.0838	0.1380
t- value	1.465	1.920	1.612
Remark	NS	NS	NS

**Table 3.2:** Comparison of carmoisine treated rats Group 1 (control) and Group 3

Parameter	TSH (mU/L)	T <sub>4</sub> (µg/dl)	T <sub>3</sub> (ng/dl)
Group 1 (0.0g/kg)	$0.2200 \pm 0.0609$	$2.185 \pm 0.4687$	$0.7933 \pm 0.6541$
Group 3 (0.11g/kg)	$0.3767 \pm 0.3051$	$2.438 \pm 0.2255$	$1.375 \pm 0.5023$
p- value	0.2456	0.2604	0.1147
t- value	1.234	1.193	1.728
Remark	NS	NS	NS

**Table 3.3:** Comparison of carmoisine treated rats Group 1 (control) and Group 4

Parameter	TSH (mU/L)	T <sub>4</sub> (µg/dl)	T <sub>3</sub> (ng/dl)
Group 1(0.0g/kg)	$0.2200 \pm 0.0609$	$2.185 \pm 0.4687$	$0.7933 \pm 0.6541$
Group 4 (0.14g/kg)	$0.1017 \pm 0.0279$	$3.012 \pm 0.4185$	$1.087 \pm 0.4628$
p- value	0.0015	0.0091	0.3909
t- value	4.323	3.222	0.8969
Remark	S	S	NS

**Table 3.4:** Comparison of carmoisine treated rats Group 1 (control) and Group 5

Parameter	TSH (mU/L)	T <sub>4</sub> (µg/dl)	T <sub>3</sub> (ng/dl)
Group 1 (0.0g/kg)	$0.2200 \pm 0.0609$	$2.185 \pm 0.4687$	$0.7933 \pm 0.6541$
Group 5 (0.18g/kg)	$0.0933 \pm 0.0378$	$3.4370 \pm 0.5896$	$1.688 \pm 0.6748$
p- value	0.0015	0.0022	0.0419
t- value	4.325	4.070	2.333
Remark	S	S	S

S = significant, NS = Not Significant

## 4. Discussion

The influence of synthetic dyes on thyroid hormones and function has drawn just but a little attention coupled with

series of controversies surrounding the effect of dyes on thyroid function. From the results obtained in this study, when group 1 and 2 as well as group 1 and 3 were compared, a non-significant increase in TSH, T<sub>4</sub> and T<sub>3</sub> was

seen. This is in line with the reports of [12], when the exposure of textile workers to azo dyes such as carmoisine was evaluated. However, this findings is contrary to the reports of [13][14][15]. They reported increase in  $T_4$  and  $T_3$  when rats were treated with carmoisine

When values of group 1 and 4 were compared, there was a significant increase and decrease of  $T_4$  and TSH respectively.  $T_3$  was increased non-significantly. The increase in  $T_4$  is in line with the findings of [13][14]. The non-significant increase in  $T_3$  is also in line with the findings of [12]. However, the decrease in TSH is contrary to the findings of [14] who reported increase in TSH levels. Finally, when group 1 and 5 were compared there was significant increase in  $T_4$  and  $T_3$  and a significant decrease in TSH levels. The increase in  $T_4$  and  $T_3$  is in line with findings of [13][14]. However, the decrease in TSH was contrary to the finding of [14] who reported increase in TSH levels.

It was revealed from the results that when the doses were increased up to 0.14g/kg and 0.18g/kg bodyweight there were significant differences in the TSH,  $T_4$  and  $T_3$  levels of the rats. It has been reported that azo dyes when oxidized by intestinal microbes (bio-transformation of azo dyes), they tend to release metabolic intermediates which are mainly reactive oxygen species and free radicals capable of causing oxidative stress and lesions on organs such as the liver [8][16][17][18][19]. The oxidative stress caused by reactive oxygen species in the liver, morphological alteration might occur resulting in the release of hepatic enzymes such as 5' de-iodinase that will enhance the peripheral conversion of  $T_4$  to  $T_3$  leading to  $T_3$  increase especially. In addition, the increase in  $T_3$  and  $T_4$  could also be as a result of reduced binding capacity of thyroid hormones carrier protein such as thyroxine binding globulin (TBG). The increase in  $T_3$  and  $T_4$  could also be as a result of direct oxidative insults from reactive oxygen species on the colloids of the thyroid glands leading the inappropriate leakage of these hormones into the plasma. The reduced TSH level could be as a result of the inverse relationship between thyroid hormones and TSH due to negative feedback control from the pituitary glands. In addition, in line with [11], the increase in TSH could be due to the effect of elevated serum iodide due to carmoisine administration rather than a direct effect on the thyroid.

## 5. Conclusion

The study suggested that oral administration of carmoisine dyes induced a dose-dependent increase in the plasma levels of  $T_3$  and  $T_4$  as well as reduced level of TSH of albino rats.

## 6. Recommendation and Future Work

This study would not be seen as conclusive but have provided a benchmark on the possible hyperthyroidism effect and reduced TSH plasma level of albino rats as a result of oral administration of carmoisine. Therefore, it is advised that more studies should be done using larger number of animals within the acceptable daily intake dose range of 0.0 - 4.0mg/kg bodyweight to determine the effect of long-term oral administration of carmoisine on  $T_3$ ,  $T_4$  and TSH.

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