



The Effect of Di-Ethylstilbestriol (DES), Oxytocin and Testosterone on the Content of Carbohydrate, Chlorophyll and Protein in Green Algae

Meghwal Rajani¹, Shekhawat Deependra Singh^{1*}, Soni Naveen Kumar¹ and Bhatnagar Chhaya²

¹Department of Microbiology, M.D.S. University, Ajmer, Rajasthan, INDIA

²Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, INDIA

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Abstract

The main aim of this study was to check the influence of sex steroids: Testosterone, Di-Ethylstilbestriol (DES) and Oxytocin on changes of proteins, chlorophyll and carbohydrate contents of green algae. Green algae were grown in BG-11 medium for 15-days period, in 4 different concentrations (1 µg/ml, 2.5 µg/ml, 5 µg/ml and 10 µg/ml) of steroids. It has been ascertained that the best possible stimulation of protein was caused by Di-Ethylstilbestriol (DES) in the range from 148.55%, testosterone 64.59% and the effect of oxytocin on protein content of green algae was negative (decreased up to 13.33%) in comparison to a 100% control. Carbohydrate content was best encouraged by testosterone in range from 42.62% in comparison to oxytocin (15.94%) while the effect of DES on carbohydrate content of green algae was negative (decreased up to 26.70%). Chlorophyll content was stimulated most by testosterone in range from 94.09%, followed by oxytocin 56.69% and DES 34.39% in contrast to 100% control during the whole period of 15 days of green algae cultivation. The effect on Protein, Carbohydrate and Chlorophyll by Testosterone ($p < 0.05$, $F_{3,88} = 61.66$, $r^2 = 0.9113$), DES ($p < 0.05$, $F_{3,88} = 41.04$, $r^2 = 0.8724$) and oxytocin ($p < 0.05$, $F_{3,88} = 491.5$, $r^2 = 0.9879$) is statistically significant.

Keywords: Carbohydrate, chlorophyll, Di-Ethylstilbestriol (DES), green algae, oxytocin, protein, testosterone.

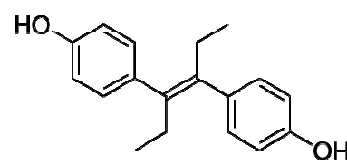
Introduction

Algae encompasses many different groups of living organisms that capture light energy through photosynthesis and convert inorganic substances into simple sugar. All algae have plastids, the bodies with chlorophyll that carry out photosynthesis, which are present in different combinations of chlorophyll molecules in varied algal strains. Some have only Chlorophyll a, some with a and b, while other strains possess a and c. Algal biomass contains three main components: proteins, carbohydrates, and natural oil. While the percentages vary with the type of algae, there are algae types that are comprised of up to 40% of their overall mass by fatty acids. It is this fatty acid that can be extracted and converted into biodiesel¹. A steroid is a terpenoid lipid, characterized by its sterane core and additional functional group. The core is a carbon structure of four fused rings: three cyclohexane rings and one cyclopentane ring. Exogenous usage of steroidal hormones in specific concentrations would enable selective growth control of plants and produced biomass could be utilized as a fertilizer in agriculture². Additional growth stimulation through exogenous and selectively used steroidal hormones seems to be advantageous in the usage of highly trophic biomass as an additive to animal feed².

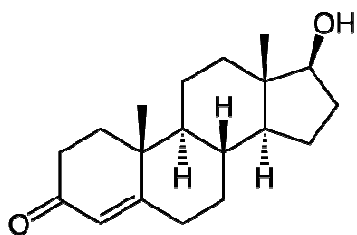
Typical male steroid hormones (androgens), mainly testosterone and its derivatives, stimulated only the

germination of seeds and the development of plants embryos in the beginning of the heterotrophic period. Androgens promoted the formation of stamens and spermatozoid production in the process of flower development. While the vegetative development of small plants begins with its photosynthesis process, it is inhibited under the influence of androgens³⁻¹⁵.

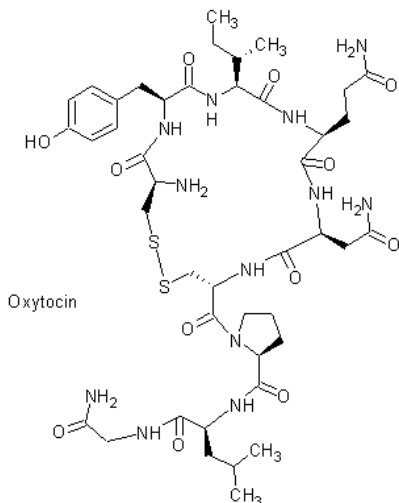
Oxytocin is best acknowledged for its roles in sexual reproduction, particularly during and after childbirth. Oxytocin is a peptide of nine amino acids (a nonapeptide). Its systematic name is cysteine-tyrosine-isoleucine-glutamine-asparagine-cysteine-proline-leucine-glycine-amine and its chemical formula is (C₄₃H₆₆N₁₂O₁₂S₂, Molecular Mass- 1007.19 g/mol). Testosterone is a steroid hormone from the androgen group. It is the principal male sex hormone and an anabolic steroid, and its chemical formula is (C₁₉H₂₈O₂, Molecular Mass- 288.4 g/mol). Diethylstilbestrol (DES) is a synthetic non steroidal estrogen that was first synthesized in 1938 (C₁₈H₂₀O₂, Molecular Mass- 268.35 g/mol).



Structure of DES



Structure of Testosterone



Structure of oxytocin

Material and Methods

The work was carried out on homogenous culture of green algae. The algae were cultivated for 15 days in 20 ml test tubes with cotton pluck stopper, containing 10 ml BG-11 medium under controlled condition (Temp. 23°C ± 1°C, photoperiod- 8 hrs. dark + 16 hrs. light). In the BG-11 medium test tube, possess the different concentration of different steroids. The pH of BG-11 medium was set to 7.0. In the experiment the steroids compound within BG-11 were applied in concentration ranges 1 µg/ml to 10 µg/ml on the 15th day of culture, when algae had reached the maximal level of development and metabolism^{6,16,17}. During 15 working days, these algae were utilized the nutrients and steroids which present in the medium for growth.

We were used the 4 different concentrations of 3 types steroids in this experiment, according to table 1 we have made the different concentrations of steroids by mix-up the specific amount of steroid stock solution with BG-11 and inoculum. The total content of biological parameters Carbohydrate, Protein and Chlorophyll were determined by specific methods such as: Porra method for Chlorophyll, Lowry method for Protein and Thio-Urea Anthrone method for carbohydrate content determination. A spectrophotometer was used for all measurements.

Stock Solution Preparation: We have required the maximum 10 µg/ml concentration of different steroids, thus we mixed the 1 ml amount of steroid into 20 ml, 0.1% Tween-80. After that we take the 1 ml of above mixer and mixed in to 99 ml sterile BG-11 medium. Right now this sample has become 10 µg/ml stock solution and this was further used for preparation of another different concentration according to table 1.

Table-1
 Different Concentrations of stock

Concentration	Steroid (stock solution)	BG-11	Inoculum
Control	0 ml	9.0 ml	1 ml
1 µg/ml	0.10 ml	8.9 ml	1 ml
2.5 µg/ml	0.25 ml	8.75 ml	1 ml
5 µg/ml	0.5 ml	8.50 ml	1 ml
10 µg/ml	1 ml	8.00 ml	1 ml

Statistical Analysis: Calculations of cluster analysis for effect of three steroids on protein, chlorophyll and carbohydrate content of green algae at 15th day (% similarity) were performed using biodiversity pro software. Other statistical analysis was performed using Prism 5.0 software. We performed one-way analysis of variance on the effect of testosterone, DES, and oxytocin on carbohydrate, protein and chlorophyll content.

Results and Discussion

Applied steroid show the greatest biological activity in green algae between 7th and 15th day of cultivation, where Testosterone and Oxytocin were more active biochemically than DES. In this experiment, four different concentrations (1.0 µg/ml, 2.5 µg/ml, 5 µg/ml and 10 µg/ml) of steroids were used for cultivation of algae and after 15 days cultivation measured the algal growth according to chlorophyll, protein and carbohydrates content in algae in comparison to control table 2.

1.0 µg/ml concentration of testosterone was negatively (decreased) effected only protein content of green algae while other concentration (2.5 µg/ml, 5 µg/ml and 10 µg/ml) were positively increased the protein, carbohydrate and chlorophyll content of green algae in comparison to control table 2.

1.0 µg/ml concentration of DES was negatively correlated (decreased) the chlorophyll content of green algae while other concentration (2.5 µg/ml, 5 µg/ml and 10 µg/ml) were positively increased the chlorophyll content and carbohydrate content concentration significantly decreased at all concentrations (1.0 µg/ml, 2.5 µg/ml, 5 µg/ml and 10 µg/ml), and protein content concentration significantly increased at all concentrations (1.0 µg/ml, 2.5 µg/ml, 5 µg/ml and 10 µg/ml) table 2.

Protein content of green algae was significantly decreased at all concentration of oxytocin (1.0 µg/ml, 2.5 µg/ml, 5 µg/ml and 10 µg/ml) and 1.0 µg/ml concentration of oxytocin was negatively affected the carbohydrate content in comparison to control table 2.

DES was maximum increased the protein content in green algae in comparison to testosterone and oxytocin, Testosterone was more increased the chlorophyll and carbohydrate content than DES and oxytocin. The effect on protein, carbohydrate and chlorophyll by testosterone ($p < 0.05$, $F_{3,88} = 61.66$, $r^2 = 0.9113$), DES ($p < 0.05$, $F_{3,88} = 41.04$, $r^2 = 0.8724$) and oxytocin ($p < 0.05$, $F_{3,88} = 491.5$, $r^2 = 0.9879$) is statistically significant.

According to Bray- Curtis cluster analysis (single link), oxytocin and testosterone were around 82.58% similar affected (either positive or negative) the protein content of green algae, DES and other two steroid were only 73.91% similar affected figure 1.

Oxytocin and testosterone were around 91.80% similarly affected the chlorophyll content of green algae, although DES and other two steroid were only 89.93% similarly affected figure 2. Oxytocin and testosterone were around 91.09% similarly affected the carbohydrate content of green algae while DES and other two steroid were only 88.70% similarly affected figure 3.

It has been ascertained that the best possible stimulation of protein was caused by Di-Ethylstilbestriol (DES) in the range from 148.55%, testosterone 64.59% and the effect of oxytocin on protein content of green algae was negative

(decreased up to 13.33%)in comparison to a 100% control. Carbohydrate content was encouraged the strongest by testosterone in range from 42.62%, oxytocin 15.94% and effect of DES on carbohydrate content of green algae was negative (decreased up to 26.70%). Chlorophyll content was stimulated mostly by testosterone in range from 94.09%, oxytocin 56.69% and DES 34.39% in contrast to 100% control during the whole period of 15 days of green algae cultivation.

Previous studies show that steroid hormones used in research were a derivative of pregnane and their work mechanics affected mainly the nucleus cell and ribosomes, chiefly the process of transcription and translation but above results in table 1 shows that only DES and Testosterone are positively influenced protein content by affecting the process of transcription and translation, but oxytocin hormone inhibit the protein synthesis in comparison to control.

In this case, the research was focused on changes of total content of proteins, carbohydrate, and chlorophyll From among used hormones, testosterone sex steroids showed stronger stimulation of carbohydrate and chlorophyll content in green algae cultivation, in comparison to analyzed DES and oxytocin. However, the content of protein was the most intensely stimulated by DES, in comparison to testosterone and oxytocin. The obtained results are some similar to data obtained from a few publications relating to research of exogenously applied sex steroids and corticosteroids on growth and biochemical changes in vascular plants: seeds, peas, beans and lentils^{5,7,15,16,17,18}.

Table-2
The influence of different concentrations of three steroids on analyzed metabolites in green algae in 7th and 15th day of cultivation

Names of steroid hormones	Applied concentration (µg/ml)	Protein concentration (mg/ml)		Chlorophyll concentration (µg/ml)		Carbohydrate concentration (mg/ml)	
		7 th day	15 th day	7 th day	15 th day	7 th day	15 th day
Control		35.25	66.66	.447	.878	35.41	69.35
Testosterone	1 µg/ml	33.04	64.12	.609	1.213	39.21	77.31
	2.5 µg/ml	38.01	73.03	.681	1.352	41.05	81.85
	5 µg/ml	49.58	97.57	.759	1.504	45.27	88.68
	10 µg/ml	56.24	109.57	.851	1.698	49.97	98.91
DES	1 µg/ml	65.35	127.00	.399	.795	32.89	64.35
	2.5 µg/ml	72.51	140.33	.473	.938	31.35	61.47
	5 µg/ml	78.64	153.33	.541	1.09	28.17	55.33
	10 µg/ml	84.08	165.66	.587	1.18	25.79	50.84
Oxytocin	1 µg/ml	34.01	65.94	.540	1.072	33.10	65.35
	2.5 µg/ml	31.25	62.71	.598	1.193	35.98	71.47
	5 µg/ml	30.58	60.13	.642	1.26	37.01	73.24
	10 µg/ml	28.49	57.80	.692	1.365	40.87	80.41

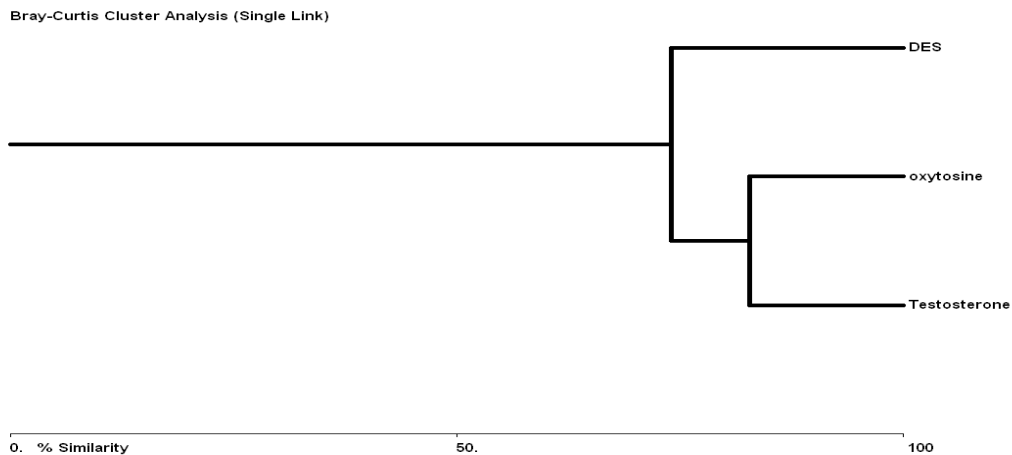


Figure-1
Effect of three steroids on protein content of green algae at 15th day (% similarity)

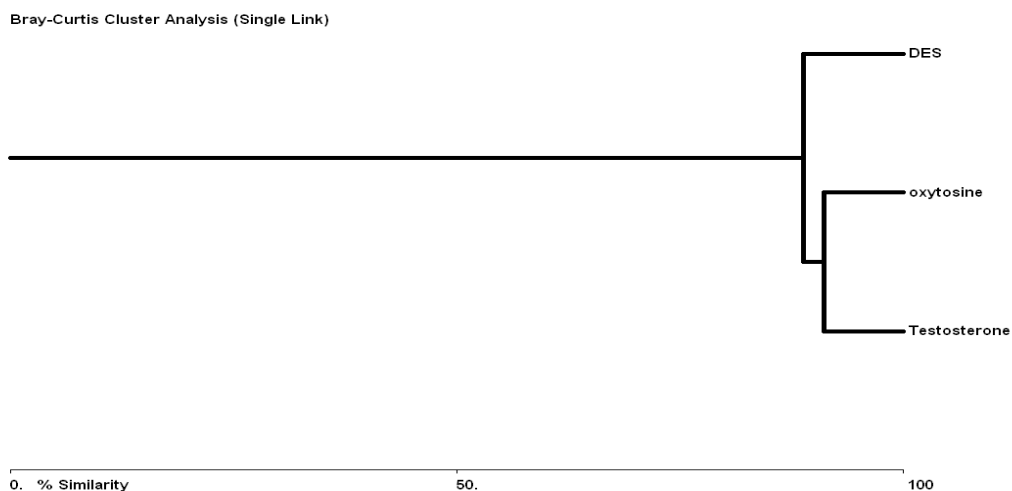


Figure-2
Effect of three steroids on Carbohydrates content of green algae at 15th day (% similarity)

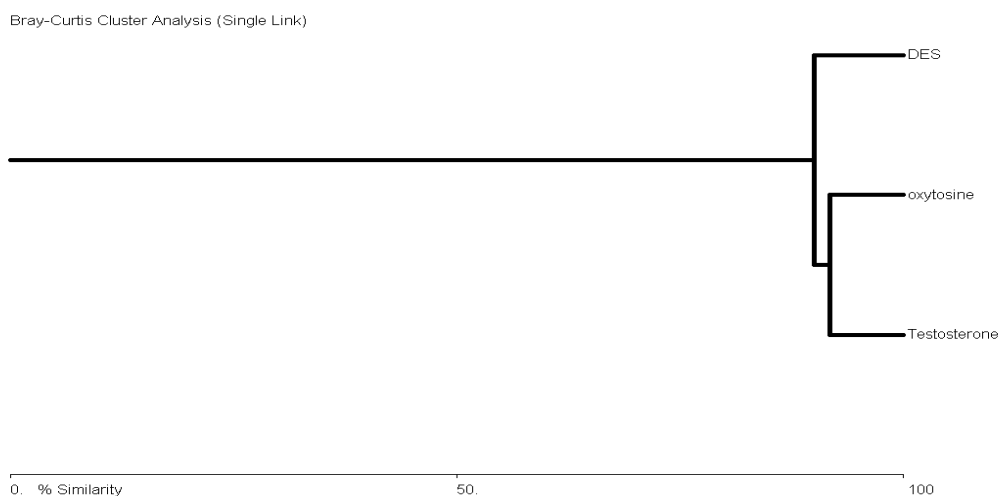


Figure-3
Effect of three steroids on Chlorophyll content of green algae at 15th day (% similarity)

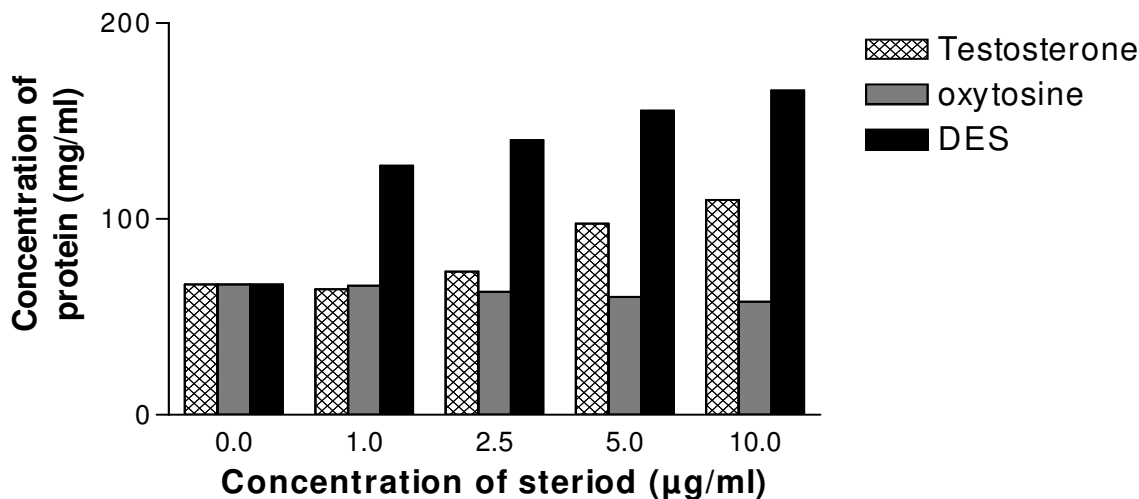


Figure-4
 Comparison of three steroids effect on protein content of green algae

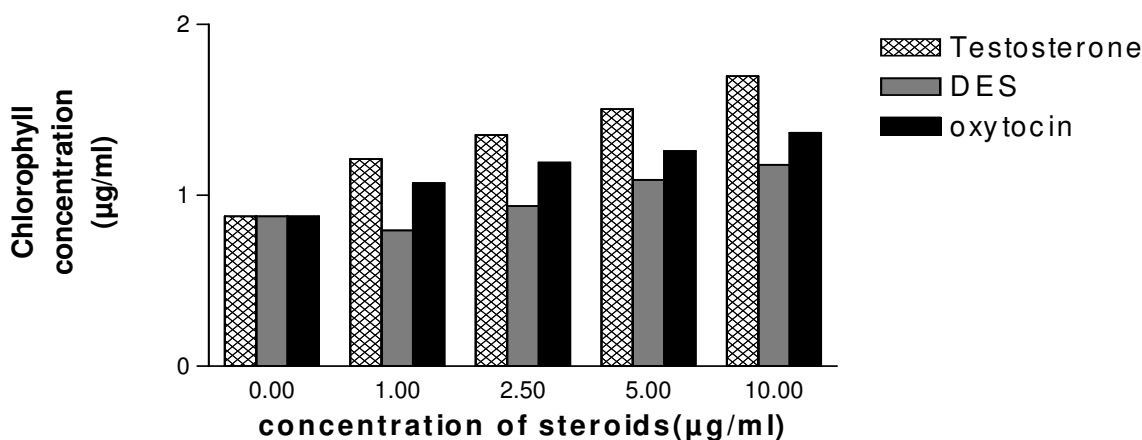


Figure-4
 Comparison of three steroids effect on chlorophyll content of green algae

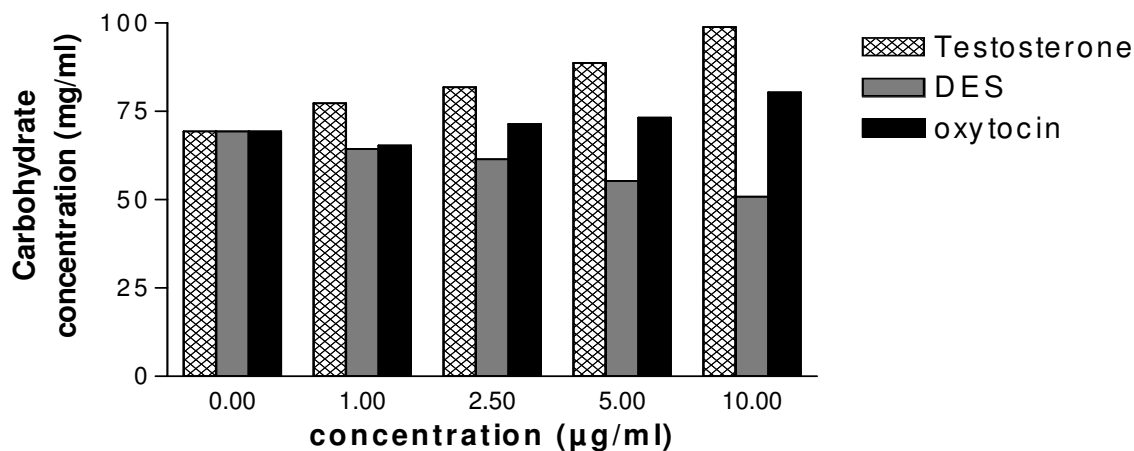


Figure-5
 Comparison of three steroids effect on carbohydrate content of green algae

Conclusion

From the above results it can be concluded that the protein content is more increase by DES. Chlorophyll and carbohydrate content are more increase by testosterone steroid. So testosterone steroid is much better for algal growth compare than DES and Oxytocin.

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