International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 05 Issue: 05 | May - 2021 ISSN: 2582-3930

Toxic effect of Amphotericin B on the Testosterone of male Swiss albino mice.

Deepak Kumar Ratan
CSIR Research Fellow, Department of zoology, A.N.College, Patna

ABSTRACT

The anti-leishmanial drug Amphotericin B is widely used in the treatment of leishmaniasis. Although itstoxic effects on testis is causing abnormalities in spermatogenesis has not been studied. But it shows spermicidal effect as well as decrease the serum testosterone level. Hence the presentstudy has been conducted to evaluate the effect if Amp B on the testosterone level. Male mice wereemployed in the study (n = 5per group). The animals were administered (i.p) with dose of Amphotericin B(6 mg/kg body weight) for 30 days and the vehicle and control treated with glucose and untreatedrespectively. Blood sample were obtained after 24 h, 2 week, 4 weeks and 6 weeks of the treatment. Mice weregiven anesthesiaand blood was collected from heart puncture. The sample was kept in appendroff and left for gravity separation of serum at 4°c. The result of this study reveals that Amphotericin B showssignificant decrease in the testosterone level.

INTRODUCTION

Amphotericin B is a polyenes antifungal drug which is used for treatment of severe fungal infections, particularly lifethreatening illnesses, including aspergillosis, cryptococcosis, systemic candidiasis, and severe cases of histoplasmosis, blastomycosis, coccidioidomycosis,Leishmaniasisandzygomycosis. Polyenes act by binding to sterols in the fungal cell membrane, forming a transmembrane channel that precipitates cell leakage and death[1].It was known to inducenephrotoxicity, cardiotoxicity but its effect on reproductive toxicity is not yet known.

MATERIALS AND METHODS

Animals were maintained under strict hygienic conditions in well ventilated rooms at 35 to 38°c and 12 hour photoperiod (0800 – 2000 hour light). All mice were housed in polypropylene cages. Rice husk was used as the bedding material. Animals were maintained on pelleted food and drinking water *ad libitum*. Male Swiss Albino Mice strain weighing (26 -36 gm) (11 -12 week old) were maintained under standardlaboratory condition in polypropylene cages. They were fed with pelleted food and water ad libitum. Fiveanimals were chosen for each group. The doses of Amphotericin b were freshly prepared 6 mg/kg bodyweight and injected (i.p) for 30 days at a time interval of 24 h and the control group received glucose anduntreated. Animals were made unconscious by using anesthesia on the 24 h, 2 week, 4 week and 6 week of the lasttreatment. The blood was collected from heart and kept at 4°c for gravity separation of serum. The serum was collected and tested for hormone assay.

Dose Chart

GROUPS	TREATMENT
G- I	Untreated Control
G- II	Vehicle treated initial control (VTIC)
G- III	24 h after drug treatment
G- IV	2 week after drug treatment
G- V	4 week after drug treatment
G- VI	6 Week after drug treatment
G- VII	Vehicle treated terminal control (VTTC)

TESTOSTERONE

Testosterone (17β-hydroxyandrost-4-ene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in C-3 and a hydroxyl group in the β position at C-17. This steroid hormone has a molecular weight of 288.4. Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig's cells of the testes. Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states.

ASSAY PROCEDURE

1. The desired number of coated wells in the holder.
2. 25 µl of standards, specimens and controls into appropriate wells.
3. 100 µl of working dilution of Testosterone-HRP Conjugate Reagent into each well.
4. 50 µl of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubated at room temperature 60 minutes.
6. Rinse and flick the microwells 3 times with 1x wash buffer water.
7. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
8. Incubate at room temperature (18-26°C) for 15 minutes.
9. Stop the reaction by adding 50µl of Stop Solution to each well.
10. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. The data was observed at 450 nm with a micro titer well reader within 15 minutes.

TESTOSTERONE

MICE	UTC	VTIC	DT 24 h	DT 2WEEK	DT 4 WEEK	DT 6 WEEK	VTTC
1	3.9	4.1	3	5	5.7	3.6	5.9
2	3.6	7.2	3.1	4.9	5.1	7.8	6.8
3	3.6	5.2	1.2	4.8	5.3	4.3	4.5
4	4.8	6	1.4	4	3.8	4.5	5
5	5.1	7.2	5.9	3.8	4.1	4.9	3.7
AVG	4.2	5.94	2.92	4.5	4.8	5.02	5.18

RESULT

The drug was given to the mice daily for 30 days at a daily dose of 6 mg/kg body weight along with appropriate vehicle, prepared fresh daily. Glucose is used as a vehicle for Amphotericin B. Measurement of hormones were made at 24 hr, 2 week, 4 week, 6 week after the 30 days of administration of dose.It is convenient to consider first thefrequency of abnormalities in all groups of control animals. The level of significance (P) value is calculatedwith the help of ANOVA analysis by SPSS 16.0the (P) value of testosterone is 0.0215.According to NEWMAN-KEUL'S the value obtained is Non-Significant within group.

Results of ANOVA analysis

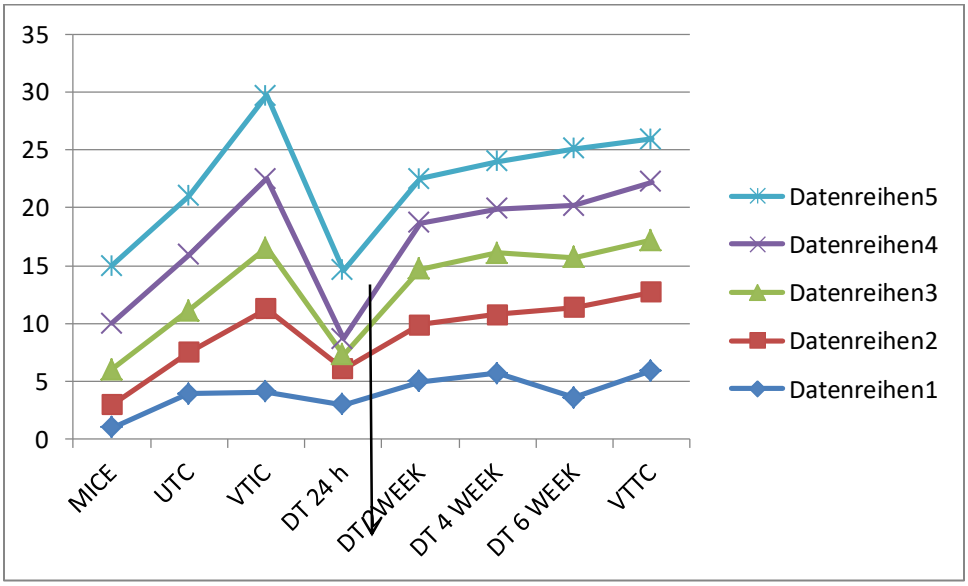
Tests of Between-Subjects Effects

Dependent Variable:VALUE

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	757.2526	1	757.2526	328.8726	5.44E-05
	Error	9.210286	4	2.302571		
TREATMENT	Hypothesis	26.61143	6	4.435238	3.101049	0.02155
	Error	34.32571	24	1.430238		
VARIABLE	Hypothesis	9.210286	4	2.302571	1.609922	0.204217
	Error	34.32571	24	1.430238		
TREATMENT * VARIABLE	Hypothesis	34.32571	24	1.430238	.	.
	Error	0	0	.c		

a. MS(VARIABLE)
b. MS(TREATMENT * VARIABLE)
c. MS(Error)

TESTOSTERONE



DISCUSSION

The results obtained by using ANOVA analysis by software SPSS 16.0. According to Swierstra et al., 1964[2,3]. It was observed that Amphotericin B results in the decrease in serum testosterone levelthe similar result has been observed in the experiment performed. It is also clear from the graph that after 30 day's treatment and autopsy done after 24 hour of the treatment the level of testosterone decreases.The drugs are likely to affect spermatogenesis and/or sperm parameters[2], the levels of scientific evidence are still insufficient.

ACKNOWLEDGEMENT

The author is grateful to Dr.Arun Kumar Singh Professor of Zoology, A.N.College Patna BiharMagadh University, India for his invaluable suggestions at different stages of this investigation.

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