

EFFECT OF HEPATITIS AND IMMUNOSTIMULANT ON SERUM TESTOSTERONE LEVEL IN EXPERIMENTAL MICE

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ABSTRACT

The present study aimed to illustrate the effect of the immunostimulant, immunex DS and IDS + hepatitis B infection on serum testosterone level in male swiss albino mice. Testosterone level was increased significantly in IDS treated mice and IDS + vaccine treated mice from day 1 to 5 of infection when compared with controls. Viral toxicity led to the abnormal increase and release of testosterone in the blood of all the experimental groups of mice due to improper spermatogenesis.

Key words: Immunex DS, Hepatitis B infection, Serum testosterone, Mice

INTRODUCTION

Hepatitis B is a major global health problem and is the leading cause of liver cancer in the world and frequently leads to cirrhosis and liver failure (Beasley *et al.*, 1981). This disease is the most common cause to increase the risk of hepatocellular carcinoma (Milich and Liang, 2003; Tong *et al.*, 2005). Hepatitis B infection causes ill-health, loss of appetite, anorexia, arthralgia, gall bladder obstruction, severe jaundice, malena condition of stools and abdominal pain. Chronic infection of hepatitis B may lead to inflammation of liver, cirrhosis and heavy fat depositions on the surface of liver (Wong and Goh, 2006; Risco *et al.*, 2009; Yeh *et al.*, 1989). The progression of liver disease and the onset of complications can be prevented by treating them with immunostimulants (by raising the resistance). Immunostimulants are natural or synthetic products showing different mechanisms of action (Marinova *et al.*, 2003; Petrunov, 2004; Petrunov *et al.*, 2007). A novel

immunostimulant immunex DS (IDS) has been employed in the present studies. IDS is a mixture of beta-carotenes, l-lysine, DI-methionine essential fatty acids, livamisol hydrochloride, vitamins like A, D₃, E, C and B₁₂ minerals like zinc, cobalt, manganese, scelenium and probiotics like bacillus and yeast. IDS boosts the natural immunity and prevents the colonization of the harmful bacteria and accumulation of toxins in gastro intestinal tract (Rao *et al.*, 2010). The currently available antiviral drugs are unable to eradicate hepatitis B virus (HBV) infection due to their inability to kill infected hepatocytes and the persistence of viral covalently cloud circular DNA (ccc DNA) in the liver of infected persons. The suppression of viral replication and disease may be possible by treating hepatitis patients with immunostimulants (by raising the resistance).

The knowledge on viral infections and their ill effects on reproductive capacity has raised medical interest as to know whether the

microbial (viral) toxins adversely effect the reproduction in humans. Serum testosterone regulates spermatogenesis and mating behaviour in males. Also, testosterone mediates male-male aggression, presumably due to its aromatization to oestrogen in the brain (Naftolin et al., 1971; Dessi-Fulgheri et al., 1976; Brain, 1983). The level of luteinizing hormone and testosterone rise during a male 'challenge' (Wingfield et al., 1990) or after exposure to a receptive female (Batty 1978; Bronson and Desjardins, 1982). Exposure of humans to environmental chemicals led to the degeneration of spermatozan and declined testosterone level (Nath and Kumar, 2007) and ovarian nuclear degeneration (Sahay et al., 2007) in mice. Endosulfan inhibited testicular function in rats (Chitra et al., 1999). Sinha et al., (1995) observed marked biochemical changes in rats treated with endosulfan. Endosulfan exposure caused declined level of testosterone due to improper spermatogenesis and degeneration of seminiferous tubule in mice (Singh et al., 2011). No information is available on the ill effects of viral infection in human animal reproduction. Therefore, the present studies are designed to understand the effect of immunex DS, and immunex DS treatment and infection with hepatitis B on the level of serum testosterone in male swiss albino mice.

MATERIALS AND METHODS

Male swiss albino mice fed with standard balanced diet and water ad libitum were taken care according to the guidelines of CPCSEA. Six groups (A, B, C, D, E and F) of each experimental mice were treated orally with a single dose of 150 mg of IDS on day 0 and waited for 72 hours, and injected intramuscularly with various single doses of Genvac B Hbs Ag vaccine (A @ 0.07 mL/mouse; B @ 0.01 mL/mouse; C @ 0.2 mL/mouse; D @ 0.4 mL/mouse; E @ 0.8 mL/mouse; F @ 1.0 mL/mouse). One group (I) of mice was treated orally (a single dose) @ 150 mg of IDS/mouse and another group (c) was kept as controls (untreated with IDS and vaccine) for comparison. From day 11 to 15, the experimental mice were sacrificed along with the

mice of IDS treated (group I) and controls (group c). Blood was collected and serum was separated and analyzed for testosterone following standard method (Winters et al., 1998). Results were analyzed following students 't' test.

RESULTS AND DISCUSSION

Results are shown in table 1. In all the experimental groups (A, B, C, D, E and F) of mice treated with immnostimulant and various doses of vaccine, it is found that the level of serum testosterone was markedly elevated from day 1 to 5 of experimental period when compared with controls. However, mice of groups B (IDS + 0.1 ml of vaccine) and F (IDS + 1 ml of vaccine) showed increased level of testosterone throughout the experimental period when compared with IDS treated (group I) animals.

Group A:

The level of serum testosterone increased significantly in mice of group A when compared with controls; but there was a significant decrease from day 1 to 5 of experimental period when compared with IDS treated mice (table 2). From day 1 (488.0 ng/dl) to 5 (502.5 ng/dl) there was gradual increase of testosterone in group A.

Group B:

In mice of group B, there was a significant increase of testosterone from day 1 to 5 when compared with controls (group c) and IDS treated (group I) mice. The increased values in group B showed a progressive rise from day 1 (626.0 ng/dl) to 5 (646.5 ng/dl) in comparison with groups c and I.

Group C:

Higher testosterone levels were found in group C from day 1 to 5 when compared with controls and lower levels of testosterone were recorded from day 1 to 5 when compared with IDS treated (group I) mice. There was a gradual increase of testosterone from day 1 (490.0 ng/dl) to 5 (500.0 ng/dl) in case of group C mice.

Group D:

Table 1. Serum testosterone (ng/dl) in control (group c), immunostimulated (Group I) and experimental (groups A, B, C, D, E and F) male swiss albino mice at different days of experimental period. Values are expressed in mean derived from 5 observations.

Days of necropsy	Group c (Control)	Group I (treated with IDS @ 150 mg/mouse only)	Group A (150 mg of IDS/mouse and infected with 0.07 ml of Hbs Ag/mouse)	Group B (150 mg of IDS/mouse and infected with 0.1 ml of Hbs Ag/mouse)	Group C (150 mg of IDS/mouse and infected with 0.2 ml of Hbs Ag/mouse)	Group D (150 mg of IDS/mouse and infected with 0.4 ml of Hbs Ag/mouse)	Group E (150 mg of IDS/mouse and infected with 0.8 ml of Hbs Ag/mouse)	Group F (150 mg of IDS/mouse and infected with 1 ml of Hbs Ag/mouse)
1	177.3	588.9	488.0	626.0	490.0	510.0	560.0	585.0
2	174.0	584.0	490.0	628.0	492.0	515.0	565.0	588.0
3	170.0	582.0	494.0	632.0	494.0	518.0	570.0	592.0
4	178.0	586.0	498.0	636.0	496.0	520.0	575.0	594.0
5	172.0	580.0	502.5	646.5	500.0	524.0	585.2	596.0

Table 2. ‘t’ values obtained in different experimental groups (A, B, C, D, E and F) of mice

Mean t-value	Experimental groups						Control groups	
	A	B	C	D	E	F	c	I
	494.4	633.7	494.4	517.4	571.0	591.0	174.2	584.1
	A c	B c	C c					
	_____	_____	_____					
	t= 28.4*	t= 23.3*	t= 48.0*					
	D c	E c	F c					
	_____	_____	_____					
	t= 34.4*	t= 15.0*	t= 52.1*					
	A I	B I	C I					
	_____	_____	_____					
	t= 7.9*	t= 2.4*	t= 13.1*					
	D I	E I	F I					
	_____	_____	_____					
	t= 6.4*	t= 0.48 [@]	t= 0.83 [@]					
	A B	A C	A D	A E	A F			
	_____	_____	_____	_____	_____			
	t= 5.5*	t= 28.4*	t= 1.5 [@]	t= 2.4*	t= 7.2*			
	B C	B D	B E	B F				
	_____	_____	_____	_____				
	t= 23.3*	t= 4.8*	t= 1.56 [@]	t= 1.95 [@]				
	C D	C E	C F					
	_____	_____	_____					
	t= 34.4*	t= 15.0*	t= 52.1*					
	D E	D F	E F					
	_____	_____	_____					
	t= 1.75 [@]	t= 6.0*	t= 0.69 [@]					

P value at 5% level of significance is 2.306

**Statistically significant values*

[@]Statistically non-significant values

The testosterone levels were found to be higher than controls (group c) and lower than IDS treated (group I) mice from day 1 to 5 of experimental period in mice of group D. Testosterone level was found to be increased gradually from day 1 (510.0 ng/dl) to 5 (524.0 ng/dl) of experimental period in mice of group D.

Group E:

In mice of group E, the testosterone values were found to be increased from day 1 to 5 of infection period and decreased from day 1 to 5 when compared with controls and IDS treated animals respectively. From day 1 (560.0 ng/dl) to 5 (585.2 ng/dl) there was a gradual increase.

Group F:

Higher level of testosterone was found in mice of group F from day 1 to 5 of infection when compared to controls and IDS treated animals. The increased level of testosterone was found to be gradual from day 1 (585.0 ng/dl) to 5 (596.0 ng/dl) in mice of group F.

All the experimental groups of mice showed a significant increase of testosterone when compared with controls. Mice of groups A, B, C and D showed significant increase of testosterone and mice of groups E and F showed a significant decrease when compared with IDS treated mice (group I). Also, there was a significant difference in the level of testosterone when the experimental groups were compared among themselves (except in between groups A and D, B and E, B and F, D and E and E and F) (Table 2).

Abnormal increase of serum testosterone in IDS (group I) treated animals reveal that IDS stimulated the animal's body reproductive mechanism thereby altering the level of testosterone and releasing in the blood stream. These results compare well with that of Nathanael and Vardhani (2014) who reported abnormal increase of liver protein in IDS treated mice. Also, it is evident that hepatitis B infection caused abnormality in the level of testosterone in all the experimental groups of mice. Testosterone level was increased to

marked extent from day 1 to 5 of infection in all the experimental groups of mice because of the toxic effect of infectious virus. This may also be due to the abnormal spermatogenesis and the destruction and degeneration of seminiferous tubules and the release of testosterone in blood. Chitra *et al.*, (1999) also postulated that testis exposed to environmental chemicals may lead to the degeneration of spermatids and abnormality in spermatogenesis. It is interesting to note that mice received IDS alone and IDS + vaccine showed increased level of testosterone when compared with controls. This explains that pre-treatment with IDS might have enhanced resistance in experimental mice of groups A, B, C, D, E and F to combat the ill effects of viral toxicity. These results compare well with that of Singh *et al.*, (2011) who reported decreased level of testosterone when treated with endosulfan only. Thus, it is clear that the administration of IDS stimulated natural immunity in experimental mice thereby inhibiting the abnormal spermatogenesis/ testosterone level to some extent. Jasmin Gold and Viveka Vardhani (2013) also reported induction of altered metabolic efficiency by the treatment of immunex DS in mice.

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