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Immunex DS induced changes in testes protein In mice

Jasmin Gold, V[1] and Viveka Vardhani, V[2]

[1-2] *Department of Zoology and Aquaculture, Acharya Nagarjuna University Nagarjunanagar-522510, (A.P.), India*

Email: vadlamudi_vv@yahoo.co.in

ABSTRACT

The present study was undertaken to assess the effect of immunostimulation and HBV antigen treatment on testes of male swiss albino mice. This study includes the protein level in the male reproductive organ. Mice treated with immunostimulant and HBV antigen showed an increase of protein compared with controls and decrease of protein compared with immunostimulated mice. Total proteins increased in the testicular tissue of immuniostimulated mice in comparission with controls. It is shown that antigen induced reproductive toxicity in male mice may be protected by treating with immuniostimulant.

Key Words: : Immunex DS, Protein , Hbs Ag, Mice

INTRODUCTION

A wide range of commercial immunostimulants are found unique in life science applications like aquaculture, poultry and livestock and animal husbandry and in medical practices for human health prospective. A novel, commercial immunostimulant is Immunex DS(IDS) which is manufactured by PVS laboratories, (A.P, India). This immunostimulant has been using in aquaculture practices particularly in shrimp culture; because of the constituents in IDS, it is selected to stimulate the immune response in higher mammalian model such as swiss albino mice (*Mus musculus albinus*). Levamisole, a potent immunostimulant stimulates macrophages and T-lymphocytes and improves cellular immunity by increasing secretion and proliferation of T-cells (Ruiz Morena *et al*., 1993; Demirci *et al*., 2005). The dried bark powder of *Terminalia arjuna* (a deciduous tree) when administered to male swiss albino mice and male wistar rats showed its anti inflammatory activity (Halder *et al*., 2009).Petrunov *et al.,* (2007) evaluated the role of immunostimulants in immunotherapy and immuno-prophylaxis; immunostimulants increase resistance to bacterial and viral infections by stimulating non-specific immunity mechanism. Immunostimulants are widely used in the field of aquaculture, poultry and livestock animals to bring a colossal revaluation in the production of food and meeting the needs of humans (Citarasu *et al*., 2006; Gelina et al .,2009; Kumar et al 2010;Thacker, 2010). The age old practices in India suggested that the use of plant products (compounds like alkaloids, flavonoids, quinones, terpenoids) show antioxidant, anti-neoplastic, anti-ulcer and anti-inflammatory properties (Dashputre and Naikwade, 2010). Jahromi *et al*., (2011) found significant increase in the concentration of LH, FSH and testosterone hormones in the serum of cinnamon treated male mice. Though vaccines against hepatitis B are available for more than a decade the infection is still reflecting the difficulties in vaccination (Alter *et al*., 1990; Shapiro and Margolis, 1990). The acute illness of hepatitis B causes liver inflammation, vomiting, jaundice and chronic hepatitis-B may eventually leads to liver cancer or cirrhosis, which responds insensitive to present chemotherapy. Vaccines are advantageous to act as therapeutic agents (Michel and Tiollais, 2010). . Severe inflammation in liver dramatically enhances the risk of hepatocellular carcinoma with a decrease in complete antioxidant activity and increase in oxidative stress and enzymatic enhancement in humans (Mastoi *et al*., 2010; Kumada *et al*., 2010).

Hepatitis B influence kidneys and reproductive organs (Zhang et al., 2010), serum cholesterol (Jasmin Gold and Viveka Vardhani, 2013) and testosterone

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Table-1. Protein (mg/ml) content in the testes of control (group N - untreated and uninfected), IDS treated (group I, @ 150 mg/mouse) and experimental (group A, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.07 ml/mouse; group B, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.1 ml/mouse; group C, treated with IDS @ 150 mg/mouse and infected with Gen Vac B vaccine @ 0.2 ml/mouse) group D, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.4 ml/mouse; group E, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.8 ml/mouse; group F, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 1 ml/mouse) male swiss albino mice at different days of experimental period. Values are expressed in the mean derived from five observation.

Day of necropsy	Group N (untreated and uninfected)	Group I (treated with IDS @150mg/ mouse and unvaccinated)	Group A (150mg of IDS/mouse and infected with 0.07ml of Hbs Ag/mouse)	Group B (150mg of IDS/mouse and infected with 0.1ml of Hbs Ag/mouse)	Group C (150mg of IDS/mouse and infected with 0.2ml of Hbs Ag/mouse)	Group D (150mg of IDS/mouse and infected with 0.4ml of Hbs Ag/mouse)	Group E (150mg of IDS/mouse and infected with 0.8ml of Hbs Ag/mouse)	Group F (150mg of IDS/mouse and infected with 1 ml of Hbs Ag/mouse)
1	115.8	312.5	320.0	160.7	166.1	156.0	176.5	116.7
2.	115.6	313.6	318.0	166.7	178.8	166.0	182.7	163.7
3.	115.0	313.8	316.0	176.9	138.7	180.0	214.4	173.5
4.	115.4	314.2	322.0	185.9	158.2	184.0	229.4	189.4
5.	115.3	313.8	324.0	220.0	130.0	190.0	287.7	204.1

(Jasmin Gold and Viveka Vardhani, 2014) and heart protein and DNA (Jasmin Gold and Viveka Vardhani, 2016 a,b). Many investigations showed marked biochemical changes in the reproductive organs of male and female mice against pesticides and hepatitis B infection (Hannesdottir et al.,2004; Bhatnagar et al., 2008, Therefore the present investigations were undertaken estimate the level of protein in testes under the influence of immunostimulant , immune DS during hepatitis in male swiss albino mice.

Materials and Methods

Eighty (8 groups,10 in each group) male swiss albino mice (*Mus musculus albinus*) (6-8 weeks; 23-31g) were employed in the present study following 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 Immunex DS (IDS) / on day 0 and waited for 72 hours, and vaccinated with various single doses of Gen Vac B Hbs Ag vaccine (A, 0.07 mL/mouse; B, 0.01mL/mouse; C, 0.2mL/mouse; D 0.4mL/ mouse; E, 0.8mL/mouse; F, 1.0mL/mouse) intramuscularly. One group (I) of mice was intubated orally with single dose of 150mg of IDS/mouse and another group (N) which was neither immunostimulated nor vaccinated served as normal controls for comparison. Later from day 11 to 15, the experimental mice were sacrificed along with the mice of IDS treated (along group I) and normal ones (group N). Samples of testes were collected, processed and analyzed for protein following method Lowry et al.(1951).

RESULTS AND DISCUSSION

Testes protein levels in all the experimental(groups A to F) were higher than controls (group N) and lower than IDS treated mice (group I) (table-1). Among the various groups of test mice, mice of group A (throughout the experimental tenure) showed a very magnifying protein content in comparison with control and IDS treated animals and among themselves. Peak level of protein was noticed on day 5 (324.0 mg/ml) in group A.

Group A: Increased level of protein was found when copmpared to controls and immunostimulated mice throughout the experimental course. Highest level of protein was noted on day 5(324.0 mg/ml).

Group B: Higher and lower level of protein was found throughout the experimental period when compared with controls (group N) and immunostimulated (group I) mice.

Group C: Increased and decreased level of protein was found during the entire the experimental period when compared to controls (group N) and immunostimulated (group I) mice. Higher level of protein was marked on day 2(178.8 mg/ml).

Group D: The mice of group D which were immunostimulated with 150 mg of IDS and vaccinate with 0.4ml/mg of Gen Vac B Hbs Ag showed increased and decreased level of protein compared to controls(group N) and IDS (group I) treated mice. A gradual increase of protein was found from day 1(156.0mg/ml) to 5(190.0 mg/ml).

Group E: Higher and lower value of protein was found in group E when compared with controls (group N) and immunostimulated (group I) mice throughout the

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

	Experimental Groups						Control Groups	
	A	B	C	D	E	F	N	I
Mean	320.0	182.0	154.3	175.2	218.1	169.4	115.4	313.5
t-value:	A N t= 161.6*	B N t=7.5*	C N t= 4.7*	D N t= 10.7*	E N t= 5.7*	F N t= 4.0*	N I t= 711.3*	
	A I t= 5.1*	B I t= 14.9*	C I t= 19.9*	D I t= 24.8*	E I t= 5.2*	F I t= 10.7*		
	A B t= 15.3*	A C t= 20.5*	A D t= 25.2*	A E t= 5.7*	A F t= 11.2*			
	B C t= 2.32*	B D t= 0.03@	B E t= 0.04@	B F t= 0.79@				
	C D t= 2.14@	C E t= 3.1*	C F t= 0.9@	D E t= 2.29@	D F t= 0.39@	E F t= 2.1@		

P value at 5% level of significance is 2.306
*Statistically significant values : Statistically non-significant values

experimental course. Highest level of protein was observed on day 5(287.7mg/ml) entire the experiment.

Group F: Increased and decreased level of protein was observed in group F when compared to controls (group N) and immunostimulated (group I) mice throughout the experiment. A gradual increase of protein from day 1 to day 5. Immunostimulated mice showed a significant increase of protein when compared with controls (group N).

Experimental animals of groups D, E and F showed a significant increase of protein when compared with controls (group N) and a significant decrease when compared with IDS treated animals (group I). Testes protein showed a significant difference in between groups D and F and a non-significant difference between groups D and E, and E and F (Table-2). The marked increase of protein in the testes of IDS administered animals (group I) demonstrated that the oral administration of immunostimulant alone stimulated the immune response in reproductive organs. Also, comparing with normals (group N), the enhanced level of protein in the testes of all the experimental groups (A, B, C, D, E, F, G and H) can reveal the protective effect of immunostimulant thereby enhancing the reproductive protein. Clark *et al.,* (2006) suggested that proteins involved in biological function may also participate in reproduction in mammals. Decrease of protein content was found in testes of male rats treated intradermally with carbendazim (a systemic fungicide) (Muthuvivegandave *et al*., 2011).

A significant increase in the protein content of testes on IDS treatment and Gene Vac B vaccine treatment may be associated with the impairment of testicular function as reported by Akbarsha *et al*., (1990) and Kaur and Parshad (1994) during selenium treatment in rat testes. Kaur *et al*., (1999) also found marked and decrease in protein content of testes in rats during selenium treatment. The present results also explain that the biochemical acitivity of the testes might be altered due to the influence of IDS and/or vaccine. Loss of balance between ROS and anti-oxidents may led to the oxidative damage of cellular macro molecules like lipids, proteins and nucleic acids and pathogenicity may also causes metabolic disfunction of almost all the vital organs (Thomas and Kalyanaraman. 1997). The direct or indirect effect of oxidative stress in testes might have

caused abnormality or in the metabolism of protein, and synthesis of testosterone in various groups of experimental mice. These observations correlate with that of Sun *et al*., (2002) who postulated that the oxidative stress in mice may cause inflammation thereby disturbing the metabolic function of the liver. Sakunthala *et al*., (2014) also opined that various doses of Gene Vac B vaccine cause enhancement in the synthesis of DNA in liver due to the viral antigen stress in mice. Mice of group A showed increased level of testes protein when compared with controls and increased level of protein when compared with IDS treated animals. Testes of mice of group B showed increased level of protein, when compared with controls and IDS treated animals. Abnormalities in testes protein are not indicative of the disease condition but the inoculated various doses of vaccine altered the capacity of the reproductive tissue responsible for the balance between protein synthesis and storage. The increased/decreased level of protein in testes of mice indicates their disturbed metabolic activity to meet the stress induced by exposure to various doses of vaccine. Kaur and Dhanju. (2004) and Dhar *et al*., (2014) also suggested that rats exposed to pesticide and fungicide exhibit enhanced metabolic activity in the plasma and testes respectively. These results compare well with that of Sinha *et al.,* (1995) who also found elevation in the activities of specific testicular marker enzymes in rats exposed to endosulfan. Esin, (2008) Hatipoglu et al., (2009) and Umar *et al.,* (2012) also explain that toxicity can cause morphological and functional changes in the male reproductive system of various animals. Muthuviveganandave *et al.*, (2011) also reported decreased protein content in testes of male albino rat exposed to low doses of carbendazim (at 12 hr and 24 hr).

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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