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THE INFLUENCE OF IMMUNEX DS AGAINST EXPERIMENTAL HEPATITIS B VACCINE ON LIVER PROTEIN AND DNA PROFILE OF MICE

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ABSTRACT

A novel drugs known as immunostimulants were developed used in adaptogenic therapies with vaccines to gain much yields in the fields of Aquaculture, Animal husbandry Poultry and live stock management. Such a novel immunostimulant is Immunex DS with a combination of vitamins, minerals, trace elements Beta glucans and probiotics. Swiss albino mice was taken as the experimental model and targeted with the Gene Vac B vaccine to induce experimental Hepatitis B condition. Three categories of mice were maintained viz a Immunex DS treated (Immunostimulated group c) , Immunex DS treated and inoculated with varied doses of Gene Vac B vaccine(groups A,B,C,D,E,and F) and controls (un treated with Immunex DS and un inoculated with Gene Vac B Vaccine) for comparison. The tissues from liver were taken and processed for the content of protein and DNA on the designated days of necropsy. And the findings throws the light on the various dimensions of the Immunex DS in disturbing/impairing the synthesis of proteins and DNA during Gene Vac B induction of pathogenic stress causing experimental hepatitis.

Key words: Immunex DS, Gene Vac B Vaccine, Swiss albino mice, Hepatitis B.

INTRODUCTION

A novel category of immunostimulants are developed during the recent decades which yielded promising panache (Blanca *et al.*, 2007) by enhancing the immune system of humans, promoting phagocytosis of foreign bodies and activating properdin and complement systems there by triggering the release of interferons (Petrunov et al., 2007). The usage of integrated immunostimulants with vaccines culminate into adjuvant therapy which reflects its applications in various vistas of Medical , Life Sciences Aquaculture , Livestock , Veterinary and Pharmaceutical sciences (Thacker, 2010 ; Gelina *et al.*, 2009). Our investigations are centered on a newer generation immunostimulant, commercially known as Immunex DS, manufactured by PVS laboratories, Andhra Pradesh in India. Immunex DS which is a complete amalgamation and a very special formulation consisting of Beta carotenes, L-lysine, DL methionine, Fatty acids, Livamisol hydrochloride, vitamins A, D3, E, C, B12, minerals like zinc, cobalt, manganese, selenium and probiotics lactobacillus, *Saccharomyces cervisiae* (Nathanael et al., 2010). Immunex DS has a profounding impact on the Physiological and metabolic aspects of the animal model. It elevates the immune response and vital energy. It also hinders the accumulation of lethal bacterial colonies in the gastro-intestinal tract. Increases the body weight

of the animal in a healthy fashion for yielding a good marketable size (Vardhani et al., 2010). The present discourse is sculptured on the use of Immunex DS in mice model (*Mus musculus albinus*) which is inoculated with commercially available Hepatitis B vaccine "Gene Vac B" to induced experimental Hepatitis B condition. Hepatitis B infection occupies the second dreadful disease of this present hour , accounting 1.5 million deaths annually by heavy risk of Hepato cellular carcinoma (HCC) , liver dysfunction , fatty liver syndrome and liver cirrhosis (Tong *et al.,* 2005 ; Mastoi *et al.,* 2010; Kumada *et al*., 2010).

Hepatitis B in acute phase causes morbid and deleterious ailments initiated by general ill health , sudden loss of hunger (Aphagia) , inflammation of the soft joints and ligaments , cystic duct abduction , heavy raise in oxidative stress , renal obstruction facilitated by Membranous glomerulo nephritis (Zhang *et al.,* 2010 ; Fabrizi *et al.,* 2010) , nausea, vomiting, tiredness, severe asthenia, and progress to severe jaundice. The chronic phase is manifested by heavy inflammation of liver, cirrhosis , fat impregnation on the surface of liver, increase in transaminases and lactate dehydrogenase (LDH) levels , increased liver Superoxide dismutase activity (Mary Chatterjee and Sil , 2006) and culminated by Hepato cellular carcinoma (Wong and Goh, 2006). The present investigations are centered on the investigations of the levels of protein and DNA in the liver of male Swiss albino mice which were treated with Immunex DS and inoculated with varied doses of Gene Vac B vaccine.

MATERIALS AND METHODS

Male Swiss albino mice (*Mus musculus albinus*) (6-8 weeks of age, Average weight 23-31g) were used in the present investigations According to the guide lines of CPCSEA(Committee for the purpose of control and supervision of experiments in animals), proper acclimatization, care , housing and hygiene were properly maintained. Eight groups of mice were maintained 10 in each group. Six groups of mice(A,B,C,D,E and F) were orally intubated with 150mg of Immunex DS with the help of a syringe fitted with a 3 inch 16gauze oral, blunt feeding needle. Later these six groups of mice were inoculated with various doses of Gen Vac B HbsAg vaccine intramuscularly. One group of mice(c) were intubated orally by giving 150mg of Immunex DS and served as immunostimulated control and another group (cc) of mice was neither immunostimulated nor inoculated served as normal controls for comparison. The mice (groups A, B ,C D, E, and F) were immunostimulated on day 0 and inoculated with different doses of Gen Vac B HbsAg vaccine on day 7 and waited for 72 hrs, later from day 11 to 15 the mice were sacrificed along with the mice of Immunex DS treated alone (group c) and normal ones (group cc). The tissues of liver were taken, macerated and well homogenized and processed for the estimation of proteins and DNA utilizing methods of Lowry *et al*. (1951) and Burton(1971).

Results were analyzed using students't' test to determine the significance

RESULTS AND DISCUSSION

Mice from all the immunostimulated and vaccinated groups (A, B, C, D, E, and F), Immunostimulated (Group c) and control (Group cc) survived till the end of experimental period. The mice of groups (D, E and F) showed lethargy, anorexia, aggressiveness, strong reluctance to feeding. Skin manifested loss of hair leaving blistered erythremas on its surface. At the final stage of experimental period mice of group B showed exopthalmous eye. The mice administered with medium to high doses of Gene Vac B (D, E and F) showed molted and malignant liver with heavy cirrhosis and signs of fatty liver syndrome (Ahmed et al., 2010). Tumors on liver surface and persistent spleenomegaly with numerous blood clots are evidently manifested. Throughout the length of the alimentary canal is inflamed in mice of group F (which is given high dose of Gene Vac B vaccine). The renal organs showed mild to heavy enlargement and necrosis which paved to chronic kidney disease and membranous

Table 1: The content of protein in liver of control (group cc), immunostimulated (group c) and experimental (groups A,B,C,D,E and F) male Swiss albino mice at different days of necropsy. Values are expressed in mean derived from five observations.

Days of necropsy	Group cc (untreated and uninnoculated)	Group c (treated with Immunex DS @ 150mg/mouse and uninnoculated)	Group A (150 mg of Immunex DS/mouse and infected with 0.07 ml of HbsAg/mouse)	Group B (150 mg of Immunex DS/mouse and infected with 0.1 ml of HbsAg/mouse)	Group C (150 mg of Immunex DS/mouse and infected with 0.2 ml of HbsAg/mouse)	Group D (150 mg of Immunex DS/mouse and infected with 0.4 ml of HbsAg/mouse)	Group E (150 mg of Immunex DS/mouse and infected with 0.8 ml of HbsAg/mouse)	Group F (150 mg of Immunex DS/mouse and infected with 1 ml of HbsAg/mouse
1	237.2	635.1	686.5	161.3	122.4	209.8	651.7	228.1
2	240.8	634.2	688.9	162.0	176.9	327.8	617.2	258.7
3	240.2	640.2	689.3	165.2	121.7	171.5	205.5	166.8
4	236.8	642.2	689.7	161.0	113.4	171.5	164.8	592.7
5	242.7	636.8	690.9	167.9	142.7	160.8	102.8	185.5

Table 2: The content of DNA in liver of control (group cc), immunostimulated (group c) and experimental (groups A,B,C,D,E and F) male Swiss albino mice at different days of necropsy. Values are expressed in mean derived from five observations.

Days of necropsy	Group cc (untreated and uninnoculated)	Group c (treated with Immunex DS @ 150mg/mouse and uninnoculated)	Group A (150 mg of Immunex DS/mouse and infected with 0.07 ml of HbsAg/mouse)	Group B (150 mg of Immunex DS/mouse and infected with 0.1 ml of HbsAg/mouse)	Group C (150 mg of Immunex DS/mouse and infected with 0.2 ml of HbsAg/mouse)	Group D (150 mg of Immunex DS/mouse and infected with 0.4 ml of HbsAg/mouse)	Group E (150 mg of Immunex DS/mouse and infected with 0.8 ml of HbsAg/mouse)	Group F (150 mg of Immunex DS/mouse and infected with 1 ml of HbsAg/mouse
1	768.8	534.4	987.7	116.6	367.3	547.3	603.3	797.3
2	768.2	531.7	991.0	116.6	327.7	319.8	597.7	556.6
3	766.7	535.5	993.5	111.1	313.6	275.5	590.0	517.7
4	765.2	533.7	995.5	105.9	240.0	243.3	552.2	592.7
5	768.1	534.7	998.8	105.5	203.6	220.0	524.4	842.7

glomerulonephritits condition. Heart is enlarged and presence of blood clots in the pericardial membrane is evident.

The content of total proteins in the liver of Immunex DS mice (group c) exhibited a high response than the controls (group cc) throughout the experimental period. Mice of groups A, D, E and F showed heightened protein response than the controls (group cc) throughout the experimental period (Table 1). Mice of group A which received lowest dose of GeneVac B vaccine showed raised protein levels throughout the experimental tenure than the normal mice (group cc) and somewhat higher than the Immunex DS treated mice (group c) alone. A noteworthy point is that the Immunex DS alone treated mice (group c/immunostimulated mice)

Table 3: 't' values obtained for liver protein in different experimental groups (A, B, C, D, E, F, G and H) of mice

Groups							
A	B	C	D	E	F	G	H
Liver Protein:							
Mean: 689.06	163.48	135.42	208.28	348.4	286.36	200.0	213.86
t value:							
A B	A C	A D	A E	A F	A G	A H	
t=675.57*	t=686.51*	t=687.59*	t=688.41*	t=688.25*	t=686.18*	t=685.43*	
B C	B D	B E	B F	B G	B H		
t=161.04*	t=162.02*	t=162.83*	t=162.67*	t=160.68*	t=159.98*		
C D	C E	C F	C G	C H			
t=134.46*	t=134.80*	t=134.69*	t=134.30*	t=134.19*			
D E	D F	D G	D H				
t=207.78*	t=207.77*	t=207.83*	t=207.80*				
E F	E G	E H					
t=348.23*	t=348.28*	t=348.27*					
F G	F H						
t=286.16*	t=286.17*						
G H							
t=199.05*							

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

showed higher protein profile than the normal mice (group cc) as well as other groups of experimental mice. On the other hand mice belonging to groups B and C manifested lower protein level than the controls. The liver protein level heightened itself reaching its prime point on day 1(651.7mg/ml) and 2 (617.2mg/ml) in mice of group E and on day 4 (592.7mg/ml) in mice of group E and on day 4 (592.7mg/ml) in mice of group F. the experimental groups (A, B, C, D, E, and F) showed statistically significant values when compared to the Immunex DS

Table 4:'t' values obtained for liver DNA in different experimental groups (A, B, C, D, E, F, G and H) of mice

Groups							
A	B	C	D	E	F	G	H
Liver DNA Mean	993.3	114.14	290.44	321.28	573.52	661.4	579.8	706.9
t value							
A B	t=989.53=*						
A C	t=991.21*						
A D	t=992.11*						
A E	t=985.59*						
A F	t=991.11*						
A G	t=989.30*						
A H	t=990.82*						
B C	t=109.08*						
B D	t=109.95*						
B E	t=103.75*						
B F	t=108.96*						
B G	t=107.19*						
B H	t=108.67*						
C D	t=289.80*						
C E	t=289.12*						
C F	t=289.18*						
C G	t=289.16*						
C H	t=289.07*						
D E	t=320.50*						
D F	t=320.44*						
D G	t=320.50*						
D H	t=320.39*						
E F	t=571.74*						
E G	t=571.30*						
E H	t=571.54*						
F G	t=660.79*						
F H	t=660.69*						
G H	t=578.47*						

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

treated and control mice. To be taken to consideration the group A mice showed higher statistically significant value than the others (Table 3).

The DNA content in the Immunex DS treated mice (group c) showed stringent decrease throughout the experimental tenure than that of normal mice (group cc) which were neither immunstumulated nor treated with GeneVac B vaccine (Table 2). The mice of group A (treated with lowest dose of GeneVac B vaccine @ 0.07ml/mouse) manifested a colossal level of DNA than the normal mice (group cc) and Immunex DS treated ones (group c), to take a note on its magnanimous orgy; the level of DNA is at its astounding zenith on day 5 (998.8mg/ml). The mice of groups B, C, and D

manifested decreased DNA content than controls (group cc) and Immunex DS treated mice (group c) throughout the experimental period. But the group F individuals treated with maximum dose of GeneVac B vaccine (1.0ml) showed increased DNA content than controls and Immunex DS treated mice on day 1 (797.3mg/ml) and 5(842.7mg/ml) . All the experimental groups showed statistically significant values , among which mice of group A showed higher statistically significant value and mice of group B (which received Immunex DS @150mg/mouse and inoculated with 0.1ml of GeneVac B vaccine) showed lowered statistically significant value (Table 4).

It is vivid from these results that administration of Immunex DS (150mg/mouse) stimulated the body's physiological mechanism there by disturbing the synthesis of proteins and DNA content. In GeneVac B inoculated and Immunex DS treated mice, the immunostimulant enabled the mice model to cope with the severe pathogenic stress during experimental hepatitis , this throws a light in the similar works of Petrunov et al (2007) and Rajendra Babu et al., (2001). The high level of proteins and DNA in the group A mice (treated with lowest dose of GeneVac B 0.07ml/mouse) indicates a slightly disturbed immune system of the host due to the shielding of Immunex DS from pathogenic challenge. The high level of proteins in the liver of experimental mice of group A would suggest that the administered Immunex DS is able to enhance the liver protein synthesis which is clearly indicated by low levels of Cholesterol on subsequent days of necropsies (Gold and Vardhani 2013).

Decreased content of DNA in groups B, C, and D would suggests that there is a severe tissue damage , severe inflammation of hepatic organ , hepatocellular carcinoma and toxicity which eventually brought forward by enhanced SOD levels (Nathanael et al., 2011 and Vivekavardhin et al, 2013) resulting in severe stress of animals and oxidation of DNA bases paving a way of double strand breakage in molecular level(Nicholls et al., (1992) and Ames (1989).

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