

INFLUENCE OF IMMUNOSTIMULANT ON ABDOMINAL MUSCLE ASPARTATE TRANSAMINASE AND ALANINE TRANSAMINASE IN MICE AGAINST HEPATITIS B

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ABSTRACT

Eight groups of male Swiss albino mice (6 - 8 weeks old; 23 - 26g wt) were employed to study the effect of Immunostimulant, (Immunex DS) on the level of abdominal muscle transaminases against Gen Vac B vaccine. In group I, Immunex DS (IDS) was orally administered (@ 150mg/mouse) to 10 mice, in 6 groups of mice (10 in each) IDS was orally administered @ 150mg/mouse on 0 day and Gen Vac B Vaccine @ 0.07 ml/mouse (group A), 0.1ml/mouse (group B), 0.2ml/mouse (group C), 0.4ml/mouse (group D), 0.8ml/mouse (group E) and 1ml/mouse (group F) was injected on day 4 of experiment. Another group (U) of mice (ten) was kept as controls for comparison (untreated with IDS + uninfected). Two mice from each experimental (A, B, C, D, E and F) and control groups U and I (after day 7 of vaccine treatment in case of experimentals) were necropsied, from day 1-5. Abdominal muscle tissue was separated and analyzed for transaminases using standard methods. Both aspartate transaminase (AST) and alanine transaminase (ALT) showed a considerable increase in all the experimental groups of mice when compared with controls (except the level of AST from day 3-5 in group F and ALT on day 5 in group A and on day 3-5 in group B) and IDS treated mice, (except the level of AST on day 1 and 2 in all the 6 groups and ALT from day 1-5 in groups A and B and on day 1 and 2 in groups C and D) during the day 1 - 30 of experimental period. The level of AST and ALT almost remained constant from day 1 to 5 of experiment in IDS treated mice. Though liver is the major organ to be influenced during toxic reactions and/or microbial infection, the process of transamination of enzymes is found to be disturbed in all the experimental groups of mice which received various doses of vaccine after immunostimulation. It is evident that IDS and/or vaccine might have caused stress resulting in the marked alteration in the level of transaminases in the abdominal muscles of mice.

Key words : ALT, AST, Abdominal muscles, Mice, Immunostimulant, Hepatitis.

INTRODUCTION

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver diseases. (Lau and Membreno, 2004). Hepatitis B virus (HBV) infects more than 300 million

people and is a major cause of acute and chronic liver diseases in the world. (Milich and Liang, 2003). The most common type of cancer which is highly associated with hepatitis B infection is reported in Asians, particularly Chinese and Indians (Wong and Goh, 2006). A large number of synthetic derivatives, inorganic compounds or

naturally occurring substances are able to depress, regulate or enhance the immune response. The immunostimulants can be employed to a new type of therapy which aims at acting on the host defense mechanisms. (Lefrancier, 1985). Immunostimulants are products from natural or synthetic origin with different characteristics and mechanism of action (Klausen et al., 1991; Petrunov, et al., 1991; Persson, et al., 1994; Marinova et al., 2000). AST and ALT activities are used as indicators of hepatocytes damage (Whitehead et al., 1999; Coppo et al., 2002; Dede et al., 2002; Asagba et al., 2004).

AST is a mitochondrial enzyme present in the liver parenchymal cells and may also be released from heart, liver, skeletal muscle and kidney (Kumar et al., 2008). Female mice infected with single doses of *Ancylostoma caninum* larvae showed a marked increase of AST and ALT (Vardhani, 1986). A significant rise in alkaline phosphatase (ALP), AST and ALT activities was found in the atherogenic diet group of rats (Naik and Sheth, 1978; Deepa and Varalakshmi, 2004). Increase of AST and ALT, superoxide dismutase, catalase and glutathione peroxidase was found in liver and kidney of ethanol treated rats (Saravanan et al., 2002). ALT, AST and ALP are considered indicators of hepatocellular health (Vozarova et al., 2002; Yang and Chen, 2003). *Withania somnifera* extract protected liver cells against oxidative stress induced by lead intoxication (Chaurasia et al., 2000) and restored the normal level of AST and ALT in dimethoate treated guinea pigs (Ju et al., 2008). Gamma irradiation caused a marked increase in serum AST and ALT levels indicating liver injury and these changes were ameliorated by using *W. somnifera* extract (Mansour and Hafez, 2012).

Significant decrease of serum proteins and increase of transaminases was found in broilers treated with Aflatoxin B1 (Madhuri et al., 2009). Administration of *Aframomum sceptrum* tends to normalize ALT, AST and ALP levels (George et al., 2010). Increased activity of AST and ALT was found in the serum of acrylamide-treated rats (Sabik, 2011). D-galactosamine/

lipopolysaccharide (D-GalN/ LPS) intoxicated rats showed a significant increase of serum AST, ALT and ALP (Fyiad et al., 2012). Thioacetamide caused elevation of serum AST and ALT in rats (Rao et al., 2014). Administration of vitamin E in combination with acrylamide significantly reduced the level of serum AST, ALT and ALP in mice (Siahkoochi et al., 2014). Oils from *Zinger officinale* and *Curcuma longa* and at a dose of 200mg/kg showed hepatoprotection by decreasing the activities of serum AST, ALT and ALP (Nwozo et al., 2014). Increased level of liver AST and ALT and abdominal muscle ALP and ACP was found in mice treated with IDS + vaccine compared to IDS treated and control animals (Sridevi and Viveka Vardhani, 2011; Madhuri and Viveka Vardhani, 2014). Though liver is the target organ to be affected by HB virus, the induced chronic hepatitis may cause much disturbances in body physiology. Therefore, the present investigations are designed to estimate the level of AST and ALT in the abdominal muscles of mice treated with IDS + HB vaccine.

MATERIAL AND METHODS

Eight groups (10 in each group) of male Swiss albino mice (*Mus musculus albinus*) (6 - 8 weeks old; 23 - 26g wt) were employed in the present study. They were fed with standard balanced diet and water *ad libitum* and taken care according to the guidelines of CPCSEA. Immunex DS (IDS) (@150mg/mouse) was given orally to one group (I) of mice (10). Another 6 groups of mice received IDS orally (@150mg/mouse) on 0 day and Gen Vac B Vaccine @ 0.07 ml/mouse in group A, 0.1ml/mouse in B, 0.2ml/mouse in C, 0.4ml/mouse in D, 0.8ml/mouse in E and 1ml/mouse in F, on day 4 of experiment. A group (U) of ten mice was kept as controls for comparison (untreated with IDS + uninfected). Two mice from each of the experimental (after day 7 of vaccine treatment) and control groups were necropsied on day 1, 2, 3, 4 and 5 of experiment, abdominal muscle tissue was separated and analyzed for transaminases following the method of Reitman and Frankel (1957). Results were analyzed for statistical significance using student's t test.

RESULTS AND DISCUSSION

Activity of AST (table 1): AST level showed considerable increase during the entire experimental period in all the experimental groups of mice (except from day 3-5 in group F) when compared with controls. In comparison with IDS treated mice, the level of AST showed increase in all the experimental groups of mice (except on day 1 and 2 in groups A, B and D, and on day 1 in groups C and E and on day 3 to 5 in group F).

Activity of ALT (table 1): ALT level showed considerable increase during the entire experimental period in all the experimental groups of mice (except on day 5 in group A and from day 3-5 in group B) when compared with controls. In comparison with IDS treated mice, the level of ALT showed increase in all the experimental groups of mice (except from day 1-5 in groups A and B, on day 1 and 2 in group C and on day 1 in group D, and on day 2 in group E).

In all the experimental groups AST showed a significant increase (except in group F) when compared with controls (group U) and decrease (except in group C) when compared with

immunostimulated mice (group I) (table 2). A significant increase of ALT was found in groups A, B, C, D, E and F (except the level of ALT in groups A, B and E when compared with controls and in group E when compared with immunostimulated mice (table 3). There was no significant difference in the level of AST and ALT in all the experimental groups when compared among themselves (except the level of AST in groups A, B, C, D and E compared with F and the level of ALT in group A when compared with groups C, D and F) in IDS treated mice (group I) The increased level of AST and ALT in IDS treated mice (group I) was found to be significant when compared with controls.

The present investigations indicate that vaccination might have brought intolerance of some inherited factors like glycogenolytic defect in the muscle (resulting in the significant increase of AST and ALT in vaccinated animals), though the role of these factors in the activation of enzymes in muscles is not known, Also, it is clear that muscle fibers changed their properties during new activities as stated by Pette and Vrbova, (1985). These results are comparable to that of Kugelberg (1976) who also reported that the adaptive mechanism which

Table 1: Alkaline phosphatase (µmoles of PNP formed/min/mg of protein) and Acid phosphatase (µmoles of PNP formed/min/mg of protein) content in abdominal muscles of experimental.

(Group A - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.07 ml/mouse), (Group B - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.1 ml/mouse), (Group C - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.2 ml/mouse), (Group D - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.4 ml/mouse), (Group E - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.8 ml/mouse), (Group F - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 1ml/mouse) and control (Group I - treated with Immunex DS @ 150 mg/mouse) (Group U - untreated and uninfected) male swiss albino mice at various days of experimental period. Values are expressed in the mean derived from 5 observations.

DN	Group A		Group B		Group C		Group D		Group E		Group F		Group I		Group U	
	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP
1	18.21	13.12	18.63	13.98	19.22	14.98	20.21	15.12	20.82	16.20	21.56	17.21	18.64	14.73	16.46	12.23
2	17.82	14.98	19.12	14.23	22.56	15.21	29.82	15.82	21.02	19.23	21.83	19.86	18.63	14.72	16.45	12.24
3	20.70	22.90	20.12	25.62	35.54	19.56	42.60	20.91	32.89	27.89	28.53	22.99	18.64	14.73	16.43	12.23
4	25.90	29.80	22.00	33.53	42.50	29.67	57.56	29.88	36.78	39.98	37.76	25.98	18.64	14.73	16.46	12.22
5	34.60	42.00	25.19	34.29	58.56	39.65	67.29	32.33	41.22	48.26	43.22	29.33	18.64	14.70	16.44	12.23

DN, Days of Necropsy ; ALP, Alkaline phosphatase ; ACP, Acid phosphatase

Table 2: t values obtained in different experimental groups (A, B, C, D, E and F) of mice

AST: Mean	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
	29.80	30.41	45.69	37.87	34.69	20.65	24.44	27.96
tvalues	A — U t = 2.45*		B — U t = 2.66*		C — U t = 2.98*			
	D — U t = 2.38*		E — U t = 3.02*		F — U t = 1.14 [⊙]			
	A — I t = 0.84 [⊙]		B — I t = 1.09 [⊙]		C — I t = 2.48*		I — U t = 193.91*	
	D — I t = 1.76 [⊙]		E — I t = 1.98 [⊙]		F — I t = 2.19 [⊙]			
	A — B t = 0.19 [⊙]		A — C t = 2.13 [⊙]		A — D t = 1.13 [⊙]		A — E t = 1.21 [⊙]	
	A — F t = 2.30*		B — C t = 2.04 [⊙]		B — D t = 1.22 [⊙]		B — E t = 1.05 [⊙]	
	B — F t = 2.43*		C — D t = 0.86 [⊙]		C — E t = 1.39 [⊙]		C — F t = 3.18*	
	D — E t = 0.48 [⊙]		D — F t = 2.63*					
	E — F t = 2.96*							

P value at 5% level of significance is 2.306. * - Statistically significant values. [⊙] - Statistically non-significant values.

exists in young rats might be altering the transamination process in experimental mice treated with immunostimulant and/or vaccine. The present observations coincide with that Vardhani (1986) and Madhuri et al., (2009) who reported significant increase in the level of serum transaminases in mice and in broilers during ancylostomiasis and aflatoxicosis. Previous study revealed that vaccination and/or immunostimulation (single dose) caused marked increase of abdominal muscle ALP and ACP in

indicating pathogenic reactions and/or injury in muscles (Madhuri and Viveka Vardhani, 2014). The present findings clearly suggest that the excessive production of free radicals and lipid peroxide might have caused the leakage of AST and ALT in muscles as suggested by Cromheecke et al., (2000). Also, the vaccine and/or the immunostimulant induced oxidative stress in muscles indicating the increase of AST and ALT level. Sridevi (2013) revealed increase of AST, ALT, ACP and ALP and

Table 3 : t values obtained in different experimental groups (A, B, C, D, E and F) of mice

ALT :	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
Mean	22.52	19.61	31.16	46.05	32.82	57.02	21.92	25.86
t values	A — U t = 0.93 [@]		B — U t = 1.04 [@]		C — U t = 2.83*			
	D — U t = 2.75*		E — U t = 2.27 [@]		F — U t = 2.99*			
	A — I t = 5.21*		B — I t = 2.80*		C — I t = 17.53*		I — U t = 327.87*	
	D — I t = 2.30*		E — I t = 1.45 ^{@#}		F — I t = 2.65*			
	A — B t = 1.25 [@]		A — C t = 2.60*		A — D t = 2.67*		A — E t = 2.11 [@]	
	A — F t = 2.92*		B — C t = 2.93*		B — D t = 2.92*		B — E t = 2.50*	
	B — F t = 3.13*		C — D t = 1.59 [@]		C — E t = 2.12 [@]			
	C — F t = 2.12 [@]		D — E t = 0.28 [@]		D — F t = 0.74 [@]			
	D — F t = 1.32 [@]		E — F t = 1.90 [@]					
	E — F t = 1.90 [@]							

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

histopathological changes in liver of mice treated with HB vaccine and/or immunostimulant as indicators of liver damage. The present results indicate that HB virus might have sensitized the experimental mouse immune system leading to the impairment in the transamination.

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