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SOD and CAT level in the abdominal muscles of immunostimulated mice during hepatitis B infection

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ABSTRACT

The effect of immunostimulant, IDS and Gen Vac B vaccine was studied on the enzymatic activities of SOD and CAT in the abdominal muscles of male Swiss albino mice mice (6 - 8 weeks old; 23 - 26g wt) at different days of experiment. Eighty mice were separated into 8 groups as group I (Immunex DS was orally administered @ 150mg/mouse on 0 day), group A (IDS @ 150 mg/mouse + 0.07 ml/mouse Gen Vac B vaccine was injected on day 4 of experiment, group B (IDS @ 150mg/mouse + vaccine @ 0.1 ml/mouse), group C (IDS @ 150mg/mouse + vaccine @ 0.2 ml/mouse), group D (IDS @ 150mg/mouse + vaccine @ 0.4 ml/mouse), group E (IDS @ 150mg/mouse + vaccine @ 0.8 ml/mouse) and group F (IDS @ 150mg/mouse + vaccine @ 1.0 ml/mouse) and group U (untreated with IDS + uninfected) was kept as controls for comparison. 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 of experiment. Abdominal muscle tissue was separated and analyzed for SOD and CAT activities using standard methods. The activity of SOD showed a considerable increase in all the experimental groups of mice and a decreased CAT activity when compared with controls (group U) and IDS treated mice (group I) during the entire experimental period. The level of SOD and CAT almost remained constant from day 1 to 5 of experiment in IDS treated mice. Increased activities of these of enzymes are responsible for regulation of active oxygen species which play a significant role in pathogenesis of muscular dystrophies. It is evident that IDS and/or vaccine might have caused stress resulting in the marked alteration in the level of SOD and CAT in the abdominal muscles of mice.

Keywords: SOD, CAT, Abdominal muscles, Mice, Immunostimulant, Hepatitis B.

INTRODUCTION

More than 300 million people worldwide are surviving as hepatitis B Virus (HBV) carriers (Lavanchy, 2004; Milich and Liang 2003). Hepatitis B is the common type of cancer which is highly reported in Asians, particularly Chinese and Indians (Wong and Goh, 2006). One way to suppress inflammatory and/or stimulation of phagocytosis is the application of immunostimulants which increase resistance to bacterial and viral infections by stimulating nonspecific immune mechanisms in mice (Petrunov et al., 2007). Certain antioxidants protect the body against the damages caused by ROS (destructive oxygen derivatives) and maintain redox homeostasis; these include both enzymatic (SOD), glutathione peroxidase (GPx), catalase (CAT), etc and non-enzymatic

(glutathione and vitamins A, E and C) antioxidants. Oxidative stress sets in when the redox balance is disrupted by extreme generation of ROS or when the antioxidant capacity is insufficient (Thomas, 2000; Golden et al., 2002). The role of oxidative stress in the mechanism of isoniazid and rifampicin-induced hepatitis has been reported by Attri et al., (2000). SOD and CAT play an important role in the biological systems to act against oxidative stress (Akyol et al., 2002). Oxygen is required to maintain life and metabolic processes including drug metabolism but ROS are generated during oxygen use (Gupta et al., 2007). It was observed that the bark extract (*Helicteres isora*) could increase the SOD and CAT activities in the cardiac tissues of diabetic rats (Kumar et al., 2008). SOD activity was decreased in both liver and kidney whereas CAT activity was increased only in liver in cadmium exposed rats and reversed on selenium administration (Ognjanovic et al., 2008). Hao et al., (2009) reported that purslane *(Portulaca oleracea)* can be used for anti-aging, thereby increasing the level of SOD and decreasing the level of MDA in the brain of mice treated with D-galactosamine. GSH homeostasis in tissues is maintained by the antioxidant enzymes such as SOD and GST (Abdel-Moneim *et al*., 2010). The hepatic antioxidant enzymatic (SOD and CAT) activites were decreased in the liver of rats administered with CCl_4, and restored by *Decalepis hamiltonii* (DHA treatment) (Srivastava and Shivanandappa, 2010). Significant acceleration in the level of lipid peroxide (MDA), with significant depletion in GSH, SOD and CAT activities in the serum of rats exposed to two different doses of gamma radiation that produced oxidative stress (Saad and Ammar, 2011). There was a significant increase of liver, kidney and testes catalase and SOD activities in purslane administered albino rats (Dkhil et al., 2011). D-galactosamine/ lipopolysaccharide (D-GalN/ LPS) intoxicated rats showed a decreased activity of hepatic antioxidants like SOD and CAT (Fyiad et al., 2012). Decreased activities of hepatic antioxidant enzymes, such as SOD and CAT were observed in isoniazide and rifampicin treated rats (Verma et al., 2013). Investigations on the hepatoprotective effects of Triphala in D-Galactosamine (D-GalN) induced hepatic toxicity in mice explain that D-GalN induced hepatic damage resulted in a significant increase in the activity of ALT, AST, ALP, bilirubin, lipid peroxidation (LPO) and Tumour necrosis factor (TNF-α) level and a decrease in the levels of anti-oxidant enzymes such as SOD, CAT, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST) and total reduced glutathione (Sabina et al., 2013). The activities of SOD and CAT were significantly lowered in tissues (liver, proximal colon, distal colon and lysate) of 1, 2-dimethylhydrazine (DMH) treated rats and linalool supplementation significantly increased the activities of SOD and CAT (Srithar *et al*., 2013). Pretreatment with *Tribulus terrestris* fruit aqueous extract (TTFAEt) maintained the normal endogeneous antioxidant activity by increasing the levels of antioxidant enzymes such as SOD and CAT in heart tissue of rats (Sailaja *et al*., 2013). Oils from *Zinger officinale* and *Curcuma longa* and at a dose of 200mg/kg showed hepatoprotection by restoring the activitiy of SOD in ethanol-treated rats (Nwozo et al., 2014). Decreased activities of CAT and SOD was observed in all the tissues of brain, liver and kidney of mice exposed to sodium fluoride (NaF) compared to control (Sandeep et al., 2014). The present investigations are carried out to understand the influence of IDS in SOD and CAT level in mice treated with Hepatitis B infection

MATERIAL AND METHODS

According to the guidelines of CPCSEA, eight groups (10 in each group) of male Swiss albino mice (*Mus musculus albinus*) (6 - 8 weeks old; 23 - 26g wt) were fed with standard balanced diet and water *ad libitum* and taken care. Immunex DS (IDS) was orally administered @150 mg/mouse to group I (10 mice). Another 6 groups of mice received IDS orally @150mg/mouse on 0 day and Gen Vac B Vaccine on day 4 of experiment; @ 0.07 ml/mouse in group A, IDS @ 150mg/mouse + 0.1ml/mouse in group B, IDS @ 150mg/mouse + 0.2ml/mouse in group C, IDS @ 150mg/mouse + 0.4ml/mouse in group D,

Table-1: Superoxide dismutase (units/mg of protein/min) and Catalase (units/mg protein), activity in abdominal muscles of experimental (Group A - treated with Immunex DS @ 150 mg/mouse and infected with HbsAg @ 0.07 ml/mouse), (Group B - treated with Immunex DS @ 150 mg/mouse and infected with HbsAg @ 0.1 ml/mouse), (Group C - treated with Immunex DS @ 150 mg/mouse and infected with HbsAg @ 0.2 ml/mouse), (Group D - treated with Immunex DS @ 150 mg/mouse and infected with HbsAg @ 0.4 ml/mouse), (Group E - treated with Immunex DS @ 150 mg/mouse and infected with HbsAg @ 0.8 ml/mouse), (Group F - treated with Immunex DS @ 150 mg/mouse and infected with HbsAg @ 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.

DN	Group A		Group B		Group C		Group D		Group E		Group F		Group I		Group U	
	SOD	CAT	SOD	CAT	SOD	CAT	SOD	CAT	SOD	CAT	SOD	CAT	SOD	CAT	SOD	CAT
1	2.11	11.89	2.54	11.09	2.92	9.62	3.21	8.93	3.86	8.56	4.28	7.23	2.89	13.88	1.89	12.88
2	1.98	12.16	2.63	10.82	3.12	10.12	3.62	9.12	4.12	7.92	6.86	6.83	2.88	13.86	1.90	12.89
3	2.45	13.18	3.45	9.21	4.16	16.18	4.82	12.80	8.23	5.78	10.12	7.98	2.86	13.89	1.88	12.86
4	3.12	19.40	3.72	8.88	5.12	20.76	8.92	16.60	10.65	5.89	16.80	9.12	2.84	13.84	1.91	12.87
5	4.11	20.19	4.10	7.65	6.18	26.98	8.96	20.10	12.89	3.34	22.60	14.60	2.87	13.85	1.87	12.85

Values are expressed in the mean derived from 5 observations.
DN, Days of Necropsy ; SOD, Superoxide dismutase ; CAT, Catalase.

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

SOD :	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
Mean	2.75	3.28	4.30	5.91	7.95	12.13	1.89	2.87
t values	A–U t = 2.21@	B–U t = 4.49*	C–U t = 3.96*					
	D–U t = 3.19*	E–U t = 3.41*	F–U t = 3.05*					
	A–I t = 0.29@	B–I t = 1.32@	C–I t = 2.33*				I–U t = 17.40*	
	D–I t = 2.41*	E–I t = 2.85*	F–I t = 2.76*					
	A–B t = 1.07@	A–C t = 2.14@	A–D t = 2.38*				A–E t = 2.86*	A–F t = 2.78*
	B–C t = 1.47@	B–D t = 2.02@	B–E t = 2.58*				B–F t = 2.62*	
	C–D t = 1.14@	C–E t = 1.92@	C–F t = 2.30@					
	D–E t = 0.93@	D–F t = 1.75@						
	E–F t = 1.10@							

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

IDS @ 150mg/mouse + 0.8ml/mouse in group E and IDS @ 150mg/mouse +1ml/mouse in group F, Another 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 sacrificed on day 1, 2, 3, 4 and 5 of experiment, abdominal muscle tissue was separated and analysed for the activities of SOD and CAT following the methods of Misra and Fridovich (1972) and Sinha (1972). Results were analysed for statistical significance using student's t test.

RESULTS AND DISCUSSION

The activity of SOD (table 1) showed a considerable increase in all the experimental groups of mice when compared with controls (except the level of SOD on day 2 in group A) and IDS treated mice (except on day 2 in group A and a same value on day 1 and 3, same value on day 1 and 2 in group B and on day 1 in group C). Whereas, a decreased CAT activity was found during the entire experimental period (except from day 3 to 5 in groups A, C and on day 4 and 5 in group D and on day 5 in group F) when compared with controls (group U) and with immunostimulated

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

	Experimental groups						Control groups	
CAT :	A	B	C	D	E	F	U	I
Mean	15.36	9.58	16.73	13.51	6.29	9.15	12.87	13.86
t values	A–U t = 1.36@	B–U t = 5.22*	C–U t = 1.17@					
	D–U t = 0.29@	E–U t = 7.17*	F–U t = 2.63*					
	A–I t = 0.82@	B–I t = 6.78*	C–I t = 0.87@				I–U t =17.99*	
	D–I t = 0.16@	E–I t = 8.25*	F–I t = 3.33*					
	A–B t = 3.02*	A–C t = 0.36@	A–D t = 0.65@				A–E t = 4.43*	A–F t = 2.68*
	B–C t = 2.18@	B–D t = 1.74@	B–E t = 2.89*				B–F t = 0.24@	
	C–D t = 0.90@	C–E t = 3.06*	C–F t = 2.12@					
	D–E t = 3.07*	D–F t = 1.68@						
	E–F t = 1.69@							

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

mice (group I) (except on day 4 and 5 in groups A, D and from days 3 to 5 in group C and on day 5 in F).

There was a significant increase in the level of SOD in all the experimental groups (except in group A) when compared with controls (group U) and with immunostimulated mice (group I) (except in groups A and B). The SOD level showed a significant increase in (group I) when compared with (group U). There was no significant difference in SOD level in all the experimental groups (except in groups A with D, E and F and B with E and F) when compared among themselves (Table 2). There was a significant increase in the level of CAT in groups B, E and F and a significant decrease in groups A, C and D when compared with controls (group U) and when compared with IDS treated mice there was a significant increase in all the groups (except in groups A, C and D). There was non significant difference in CAT level in all the experimental groups (except in groups A with B, E and F and B, C, D with E) when compared among themselves (Table 3).

The present investigations indicate that vaccination might have brought intolerance of some inherited factors like glycogenolytic defect in the muscle, 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).

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