Biolife 3(1):26-30
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

GST level in the abdominal muscles of mice treated with Immunex DS and GeneVac B vaccine

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ABSTRACT

The effect of immunostimulant, Immunex DS (IDS) and Gen Vac B vaccine was tested against GST in the abdominal muscle of male Swiss albino mice (6 - 8 weeks old; 23 - 26g wt) at different days of experiment. Six groups of experimental mice (A, B, C, D, E and F), one control group (U) and another IDS treated group (I) were selected. IDS was orally administered @ 150mg/mouse on 0 day (group I). To all the mice of groups A to F, IDS was orally administered (@ 150mg/mouse) on 0 day and Gen Vac B vaccine was inoculated on day 4 of experiment (0.07 ml/mouse in group A, 0.1ml/mouse in group B, 0.2ml/mouse in group C, 0.4ml/mouse in group D, 0.8ml/mouse in group E, 1.0ml/mouse in group F). Group U was untreated (with IDS) and 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 GST activity using standard method. Increased activity of GST might be due to the 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 GST level in the abdominal muscles of mice.

Keywords: GST, Abdominal muscles, Mice, Immunostimulant, Hepatitis B.

INTRODUCTION

Hepatitis B virus (HBV) infects more than 300 million people and is a major cause of acute and chronic liver disease in the world (Milich and Liang, 2003). HBV is a major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver diseases (Lau and Membreno, 2004). Hepatitis B is the common type of cancer which is highly reported in Asians, particularly in Chinese and Indians (Wong and Goh, 2006). Synthetic derivatives, inorganic compounds or naturally occurring substances and/or immunostimulants are able to depress, regulate or enhance the immune response (Lefrancier, 1985). Both enzymatic (SOD), glutathione peroxidase (GPx), catalase (CAT), etc and non-enzymatic (glutathione and vitamins A, E and C) antioxidants protect the body against the damages caused by ROS (destructive oxygen derivatives) and maintain redox homeostasis. 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). In addition to SOD and CAT, the GSTs are important in oxidative stress response. Silymarin, a western herb showed a protective action against glutathione depletion (Mira et al., 1994) and iron overload (Pietrangelo et al., 1995) in rats against lipid peroxidation (antioxidant activity) and in rat hepatocytes (Carini et al., 1992). Mainwaring

et al., (1996) reported significantly higher levels of GST in mouse tissues than in rat or human tissues. GST play an important role in the biological systems to act against oxidative stress (Akyol *et al*., 2002). Decreased activity of GST was seen in lung cancer bearing mice, where as Quercetin supplementation attenuated all these alterations (Kamaraj et al., 2007). The decreased activity of GST in heart tissues was brought back to near normal upon *Helicteres isora* (HI) treatment which possesses promising antioxidative activity in streptozotocin diabetic rats (Kumar et al., 2008). GST activity was increased in both liver and kidney in cadmium exposed rats and reversed on selenium administration (Ognjanovic et al., 2008). Pretreatment of *Decalepis hamiltonii* (DHA) increased the GST activity in the liver of rats administered with carbon tetrachloride (CCl_4) (Srivastava and Shivanandappa, 2010). Two doses of Clarithromycin (Claricin) significantly ($p<0.05$) reduced the activities of hepatic GST by 23 and 36% respectively in the plasma of rats (Olayinka and Ore, 2012). A significant decrease in myocardial GSH level along with decrease in the activities of glutathione dependent enzymes (GPx, GST) was found in heart tissue of isoproterenol (ISO) administered rats, and a pretreatment with *Tribulus terrestris* fruit aqueous extract (TTFAEt) restored the normal levels (Sailaja *et al*., 2013). The activity of GST was significantly decreased in the liver and colon mucosa of 1, 2-dimethylhydrazine (DMH) alone treated rats, while linalool supplementation enhanced the GST activity (Srithar *et al*., 2013). Oils from *Zinger officinale* and *Curcuma longa* at a dose of 200mg/kg showed hepatoprotection by restoring the activity of GST in ethanol-treated rats (Nwozo *et al*., 2014). GST activity was increased in all the tissues (brain, liver and kidney) of mice exposed to sodium fluoride (NaF) compared to control (Sandeep et al., 2014). Madhuri and Viveka Vardhani (2014) found significant alteration in the level of SOD and CAT in the abdominal muscles of mice under the influence of immunostimulant, IDS and vaccine. Therefore, the present investigations are designed to estimate the GST content in the abdominal muscles of mice treated with IDS and vaccine or IDS.

MATERIAL AND METHODS

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 according to the guidelines of CPCSEA. Eight groups (10 in each group) were employed in the present study. The Immunex DS (IDS) (@150mg/mouse) was orally administered to group (I) (ten mice). 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 group B, 0.2ml/mouse in group C, 0.4ml/mouse in group D, 0.8ml/mouse in group E and 1ml/mouse in group F on day 4 of experiment. Group U (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 activity of GST following the method of Habig et al., (1974). Results were analysed for statistical significance using student's t test.

RESULTS AND DISCUSSION

The activity of GST (Table 1) showed a considerable increase in all the experimental groups of mice when compared with controls (group U) (except on day 1 in groups A and C). There was a gradual increase of GST level in groups A (from day 4 to 5), B (from day 3 to 5), C (from day 2 to 5), E (from day 3 to 5) and F (from day 3 to 5). When compared with immunostimulated mice (group I).

There was a significant increase of GST in experimental groups C, E and F) when compared with controls and there was a significant increase in group C when compared with immunostimulated mice (group I) (Table2). There was non significant difference in the level of GST in all the experimental groups when compared among themselves (except in between groups A and C, B and C, C and D, and D and F).

Table 1: Glutathione S-transferase (nanomoles GS-CDNB formed/mg protein/min) activity in the abdominal muscles of experimental (Group A - treated with IDS @ 150 mg/mouse and infected with Hbs Ag @ 0.07 ml/mouse, Group B - treated with IDS @ 150 mg/mouse and infected with Hbs Ag @ 0.1ml/mouse, Group C - treated with IDS @ 150 mg/mouse and infected with Hbs Ag @ 0.2ml/mouse, Group D - treated with IDS @ 150 mg/mouse and infected with Hbs Ag @ 0.4ml/mouse, Group E - treated with IDS @ 150 mg/mouse and infected with Hbs Ag @ 0.8ml/mouse, Group F - treated with IDS @ 150 mg/mouse and infected with Hbs Ag @ 1.0ml/mouse) and control (Group I - treated with IDS @ 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 GST	Group B GST	Group C GST	Group D GST	Group E GST	Group F GST	Group I GST	Group U GST
1	52.69	54.12	52.21	56.12	56.23	58.23	57.48	52.43
2	53.87	54.36	65.63	55.93	57.12	57.93	57.46	52.42
3	54.55	58.23	78.97	47.89	62.45	62.21	57.43	52.40
4	59.41	59.12	86.96	30.44	89.24	72.88	57.50	52.41
5	62.68	59.34	94.34	20.55	96.34	85.76	57.49	52.38

DN- Days of Necropsy, GST – Glutathione S-transferase

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

GST :	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
Mean	56.64	57.03	76.22	42.18	72.27	67.40	52.40	57.47
t values	A–U t = 2.24@	B–U t = 2.19@	C–U t = 3.36*					
	D–U t = 1.42@	E–U t = 2.33*	F–U t = 2.81*					
	A–I t = 0.43@	B–I t = 0.38@	C–I t = 2.64*				I–U t = 80.16*	
	D–I t = 2.13@	E–I t = 1.74@	F–I t = 1.84@					
	A–B t = 0.17@	A–C t = 2.66*	A–D t = 1.95@				A–E t = 1.79@	A–F t = 1.90@
	B–C t = 2.67*	B–D t = 2.05@	B–E t = 1.77@				B–F t = 1.90@	
	C–D t = 3.38*	C–E t = 0.35@	C–F t = 0.99@					
	D–E t = 2.06@	D–F t = 2.82*						
	E–F t = 0.48@							

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

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 enzyme 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).

ACKNOWLEDGEMENTS

The author (Madhuri, D) is thankful to UGC, New Delhi for financial assistance in the form of RGNF and to Prof. PVV Satyanarayana, the then Head of the Department of Biochemistry for providing laboratory facilities.

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DOI:
https://dx.doi.org/10.5281/zenodo.7262881
Received: 13 January 2015;
Accepted; 27 February 2015;
Available online : 9 March 2015