



## Total protein, DNA and RNA content in the abdominal muscles of mice treated with Immunex DS and Hepatitis B Vaccine

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### ABSTRACT

Total protein, DNA and RNA was estimated in the abdominal muscles of experimental and control male swiss albino mice (6-8 wks old; 23-26 g wt). Immunex DS (IDS) was orally administered (@150mg/mouse) as a single dose in mice of group I. In a set of 6 experimental groups, IDS was orally administered @150mg/mouse in a single dose on day 0 and injected with Gene Vac B vaccine on day 4 of experiment (@ 0.07ml/mouse, A; 0.1ml/mouse, B; 0.2ml/mouse, C; 0.4ml/mouse, D; 0.8ml/mouse, E, and 1ml/mouse, F). A single group was kept as control (untreated and uninfected). Two mice from all the eight groups were sacrificed on day 8, 9, 10, 11 and 12 after vaccine treatment. Muscle tissue (abdominal) was separated and analysed for total protein, DNA and RNA using standard methods. The level of protein, DNA and RNA showed much alteration in all the experimental groups of mice throughout the infection period when compared with controls and IDS treated mice; this abnormality might be due to IDS and/or pathogenic stress caused by reaction oxygen species.

Key words: Protein, DNA, RNA, Abdominal muscles, mice, Immunostimulant, Hepatitis.

### INTRODUCTION

The nonspecific resistance of the host can be enhanced with the use of immuno-stimulant; they modulate the complex network of reactions operating within the immune system (Jolles and Werner, 1981). The biologically active substances comprising of drugs and nutrients (immuno-stimulants) are widely used in the modulation of

immune responses in many animal models. Immuno-stimulants suppress inflammation and/or stimulate phagocytosis thereby increasing resistance to bacterial and viral infections in mice (Petrunov et al., 2007). Hepatitis B virus (HBV) infection is a major global public health problem (Szabo et al., 2003) and chronic hepatitis leads to the development of cirrhosis and liver cancer (Tiollais et al., 1985; Shih et al., 1996). Two billion people are infected with HBV (WHO, 2005) and among them 350 - 400 million are chronic HBV carriers and about 1 billion people deaths occur annually due to HBV related liver failure, cirrhosis and hepatocellular carcinoma (Feng-min and Hui, 2009). Universal vaccination to HBV infection has been implemented in 168 countries worldwide which resulted in substantial decrease in disease burden, in the carrier rate and in the hepatitis B related morbidity and mortality (Zanetti et al., 2008). Proteins play a vital role in body metabolism dealing with the essential fabric of

#### How to cite this article:

Divya Teja, D and VivekaVardhani, V (2015). Total protein, DNA and RNA content in the abdominal muscles of mice treated with Immunex DS and Hepatitis B Vaccine. Biolife, 3(2), pp 469-475. doi:10.17812/blj2015.32.16

Published online: 14<sup>th</sup> May, 2015

growth, repair and reproduction and with other dynamic expressions of vital activity involving continuous chemical change such as the maintenance of the osmotic relationships in the tissue fluids, immunity mechanisms, enzyme systems and hormones (Cuthbertson, 1948). All animal species, given a low protein diet, reproducibly and predictably failed to grow normally; and with time, become nutrition dwarfs (Malcolm, 1970). During the early phase of critical illness the body's priority is central protein synthesis at the expense of protein loss from the skeletal muscle compartment, which normally accounts for approximately 80% of the total lean tissue mass (Daniel, 1977). Protein plays a major role in the synthesis of microsomal detoxifying enzymes and to detoxify the toxicants in the animal body (Kelly, 1986). Evolutionary or functional relationships can be determined by the alignment of a set of related DNA, RNA or protein sequences (Hertz and Stormo, 1999).

Regeneration of the remnant liver is characterized by increased DNA synthesis of normally quiescent hepatocytes (Michalopoulos and DeFrances, 1997; Fausto, 2000). Proliferation of tissue *in vivo* can be evaluated by DNA synthesis (Ueda et al., 2005). Acrylamide toxicity in rats showed that hepatic tissue produces large amounts of free radicals such as Reactive Oxygen Species (ROS) which mediate tissue damage resulting in alterations in the cellular macromolecules such as membrane lipids, DNA, and proteins (Kehrer et al., 1990; Islam et al., 2012). There was a significant inhibition in DNA, RNA, and protein content in liver tissue of galactosamine/lipopolysaccharide treated rats (Fyiad et al., 2012). RNAs play an integral part in all kingdoms of life and mediate critical processes from gene regulation to genomic maintenance and protein synthesis (Liszewski, 2013).

Administration of anti-tubercular drugs (Isoniazid and Rifampicin) induced alterations on protein metabolism and hepatic antioxidant defense system; they were normalized by *Cissampelos pareira* co-administration, indicating a possible cytoprotective role of *C. pareira* against drug induced hepatotoxicity (Verma and Hussain, 2013). Thiacetamide induced liver toxicity leads to decrease of total protein and albumin level due to tissue damage. However, treatment with *Lannea coromandelica* plant extracts increased the serum protein and albumin level indicating

hepato-protective activity (Rao et al., 2014). Since many viral and bacterial diseases induce alterations in the protein, DNA and RNA constituents in various animal tissues, the present investigation are designed to estimate the level of protein, DNA and RNA in the abdominal muscles of mice during immune-stimulation and vaccination.

## MATERIAL AND METHODS

Six to eight weeks old (23 to 26 g wt) male Swiss albino mice (*Mus musculus albinus*) were used (eight groups, 10 in each) in the present work; they were fed with standard balanced diet and water *ad libitum* and were cared following the guidelines of CPSCEA. Immunex DS (IDS) was given (@150mg/mouse) with a syringe fitted with a blunt oral feeding needle to all the 7 groups (A, B, C, D, E, F and I) of mice. Gene Vac B vaccine (@ 0.07 ml/mouse, 0.1 ml/mouse, 0.2 ml/mouse, 0.4ml/mouse, 0.8ml/mouse and 1ml/mouse) was given to mice of groups A, B, C, D, E and F respectively on day 4 of experiment. Another group (U) (ten) of mice served as controls (untreated with IDS + unvaccinated). Two mice from each experimental and control groups were sacrificed on day 1, 2, 3, 4 and 5 of experiment (7 days after vaccine injection), abdominal muscle tissue was separated and total protein, DNA and RNA content was estimated following the methods of Lowry et al., (1951), Burton (1956) and Ceriotti (1955). Results were analysed for statistical significance using Student's t test.

## RESULTS AND DISCUSSION

Protein, DNA and RNA levels showed considerable increase from day 1 to 5 of experimental period in mice of group I (which received IDS only) when compared with controls; the increased values of protein, DNA and RNA almost remained constant from day 1 to 5 (Table 1 and 2). Experimental mice which received various doses of HB vaccine along with immune-stimulant showed remarkable changes in the estimated values of protein, DNA and RNA. It is of interest to note that mice which received low dose (group A @ 0.07 ml/mouse) of vaccine showed a gradual decrease in the content of protein from day 1 to 5 (except on day 1 and 2 when compared with mice of group U) with a peak value on day 1.

**Table 1. Total protein (mg/100mg), DNA ( $\mu\text{g}/100\text{mg}$ ), RNA ( $\mu\text{g}/100\text{mg}$ ) content in the 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) 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 (treated with 150 mg of Immunex DS/mouse and infected with Hbs Ag @ 0.07 ml/mouse)			Group B (treated with 150 mg of Immunex DS/mouse and infected with Hbs Ag @ 0.1 ml/mouse)			Group C (treated with 150 mg of Immunex DS/mouse and infected with Hbs Ag @ 0.2 ml/mouse)			Group I (treated with 150 mg of Immunex DS/mouse)			Group U (untreated and uninfected)		
	P	D	R	P	D	R	P	D	R	P	D	R	P	D	R
1	24.62	192.0	358.0	25.16	221.0	398.0	26.12	268.0	425.0	25.50	195.5	345	23.00	182.0	339.0
2	24.12	206.0	372.0	25.41	248.0	412.0	38.53	276.0	452.0	25.51	195.4	346	23.01	183.0	336.0
3	20.40	220.0	400.0	26.12	258.0	436.0	58.87	321.0	500.0	25.53	195.3	347	22.99	184.0	338.0
4	18.30	275.0	437.0	29.80	296.0	485.0	89.67	435.0	545.0	25.50	195.5	345	22.92	182.0	339.0
5	14.90	300.0	442.0	31.20	350.0	497.0	92.43	567.0	600.0	25.52	195.5	345	23.00	182.0	335.0

DN, Days of Necropsy; P, Protein; D, DNA; R; RNA

The level of DNA and RNA increased from day 1 to 5 of experiment (except the content of RNA on day 1) with a peak value on day 5. In mice of group B, there was a gradual increase in the total protein, DNA and RNA contents from day 1 to 5 of experiment (except the protein content on day 1 and 2); with a peak value of protein, DNA and RNA on day 5. Mice of group D (received vaccine @ 0.4 ml/mouse) showed a gradual increase in the DNA and RNA contents (from day 1 to 5), whereas the level of protein increased on day 1 and 2 (with a peak value of protein on day 2) and showed a decreased level on day 3, 4 and 5 and DNA and RNA on day 5 of experiment. Mice of group C (@ 0.2 ml/mouse), D (@ 0.8 ml/mouse) and F (@ 1.0 ml/mouse) showed a gradual increase in the content of total protein, DNA and RNA throughout the experimental period (from day 1 to 5) and reached peak values on day 5 of experiment when compared with control (U) and immunostimulated (group I) mice. Administration of three different single doses and multiple doses of *Ancylostoma caninum* larvae in mice stimulated the immune response in stomach with regard to the significant alterations in the level of protein, aminoacids, DNA and RNA (Viveka Vardhani and Sakunthala, 2011) and oxidative stress in liver (Viveka Vardhani, 2012) during *A. caninum* in mice.

Significant increase of protein was found in mice treated with IDS along with vaccine @ 0.1ml (group B) and 0.8 ml (group E) when compared with controls. It is of interest to note that mice

received 0.2ml (group C) and 1.0ml (group F) of vaccine (pretreated with IDS) showed significant increase of protein when compared with controls and IDS treated mice (Table 3). DNA and RNA increased significantly in all the experimental groups when compared with control, IDS treated and among themselves (with few exceptions) (Table 4 and 5). Dukan and Nystronm, (1999) suggested that the ROS may react with protein and/or DNA leading to their denaturation. The increase or decrease of protein might be due to modulation in cell functions or damage in cellular constituents like protein and DNA. The increase in the protein level suggest that the abnormal physiological changes caused by various single oral doses of immunostimulant against vaccine are in correlation with Vinod Kumar and Vardhani (2013) who also reported abnormal protein, DNA and RNA metabolism in liver of mice exposed to pathogenic stress. Kasai, (1997), Beckman and Ames (1997); Tarakalakshmi, Y and Viveka Vardhani (2014) and Inoue et al., (2003) opined that oxygen required for energy metabolism in aerobic organisms may generate ROS to impair protein, DNA and lipid. These results compare well with that of Sie (1985) who suggested intensive proteolysis in mammalian tissue due to pathogenic stress. The alteration in the level of protein, DNA and RNA in the abdominal muscles of mice treated with immunostimulant alone (group I) and in those pretreated with immunostimulant and then with vaccine (groups A to F) suggest that the IDS was successful in boosting the immune response.

**Table 2.** Total protein (mg/100mg), DNA (µg/100mg), RNA (µg/100mg) content in the abdominal muscles of experimental (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 D (treated with 150 mg of Immunex DS/mouse and infected with Hbs Ag @ 0.4 ml/mouse)			Group E (treated with 150 mg of Immunex DS/mouse and infected with Hbs Ag @ 0.8 ml/mouse)			Group F (treated with 150 mg of Immunex DS/mouse and infected with Hbs Ag @ 1 ml/mouse)			Group I (treated with 150 mg of Immunex DS/mouse)			Group U (untreated and uninfected)		
	P	D	R	P	D	R	P	D	R	P	D	R	P	D	R
1	27.12	293.0	438.0	28.14	289.0	483.0	30.28	392.0	569.0	25.50	195.5	345	23.00	182.0	339.0
2	37.82	312.0	469.0	28.92	329.0	516.0	31.23	412.0	592.0	25.51	195.4	346	23.01	183.0	336.0
3	12.98	478.0	523.0	39.48	424.0	533.0	46.74	577.0	623.0	25.53	195.3	347	22.99	184.0	338.0
4	16.60	566.0	706.0	69.78	476.0	587.0	73.90	896.0	639.0	25.50	195.5	345	22.92	182.0	339.0
5	20.10	728.0	748.0	90.00	540.0	627.0	81.67	634.0	656.0	25.52	195.5	345	23.00	182.0	335.0

DN, Days of Necropsy; P, Protein; D, DNA ; R, RNA.

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

Protein Mean	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
	20.46	27.53	61.12	41.05	51.26	52.76	22.98	25.51
t values	A — U t = 1.32 <sup>®</sup>		B — U t = 3.47*		C — U t = 2.86*			
	D — U t = 1.42 <sup>®</sup>		E — U t = 2.30*		F — U t = 2.78*			
	A — I t = 2.70*		B — I t = 1.92 <sup>®</sup>		C — I t = 2.70*			
	D — I t = 1.25 <sup>®</sup>		E — I t = 2.12 <sup>®</sup>		F — I t = 2.58*			
	A — B t = 3.06*		A — C t = 3.01*		A — D t = 1.59 <sup>®</sup>		A — E t = 2.46*	A — F t = 2.95*
	B — C t = 2.51*		B — D t = 1.06 <sup>®</sup>		B — E t = 1.92 <sup>®</sup>		B — F t = 2.34*	
	C — D t = 1.09 <sup>®</sup>		C — E t = 0.54 <sup>®</sup>		C — F t = 0.48 <sup>®</sup>			
	D — E t = 0.57 <sup>®</sup>		D — F t = 0.71 <sup>®</sup>					
	E — F t = 0.09 <sup>®</sup>							

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

**Table 4. t values obtained in different experimental groups (A, B, C, D, E and F) of mice**

DNA Mean	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
238.60	274.60	373.40	475.40	411.60	582.20		182.60	195.40
t values	A — U t = 2.68*		B — U t = 4.11*		C — U t = 3.65*			
	D — U t = 3.58*		E — U t = 4.95*		F — U t = 4.38*			
	A — I t = 2.07@		B — I t = 3.54*		C — I t = 3.40*			
	D — I t = 3.44*		E — I t = 4.68*		F — I t = 4.24*			
	A — B t = 1.17@		A — C t = 2.39*		A — D t = 2.82*		A — E t = 3.41*	A — F t = 3.67*
	B — C t = 1.73@		B — D t = 2.38*		B — E t = 2.67*		B — F t = 3.27*	
	C — D t = 1.05@		C — E t = 0.54@		C — F t = 3.49*			
	D — E t = 0.68@		D — F t = 0.87@					
	E — F t = 1.66@							

**Table 5. t values obtained in different experimental groups (A, B, C, D, E and F) of mice**

RNA Mean	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
401.80	445.60	504.40	576.80	549.20	615.80		337.40	345.60
t values	A — U t = 3.82*		B — U t = 5.51*		C — U t = 5.31*			
	D — U t = 3.79*		E — U t = 8.23*		F — U t = 17.55*			
	A — I t = 3.33*		B — I t = 5.10*		C — I t = 5.02*			
	D — I t = 3.73*		E — I t = 7.91*		F — I t = 17.06*			
	A — B t = 1.70@		A — C t = 2.87*		A — D t = 2.72*		A — E t = 4.79@	A — F t = 9.26*
	B — C t = 1.57*		B — D t = 2.01@		B — E t = 3.20*		B — F t = 6.75*	
	C — D t = 1.03@		C — E t = 1.10@		C — F t = 3.15*			
	D — E t = 0.40@		D — F t = 0.60@					
	E — F t = 2.20@							

Foot Note of Table-4 & 5: P value at 5% level of significance is 2.306. \* - Statistically significant values. @ - Statistically non –significant values.

However, compared with group I, immunostimulation in experimental groups (A to F) did not reflect much protection against vaccination. Further it can be concluded that mice exposed to IDS and/or vaccination undergone stress thereby indicated abnormality in the synthesis of protein, DNA and RNA.

## Conflict of Interests

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

## Acknowledgements:

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

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