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Influence of Gene Vac B vaccine on the abdominal muscle MDA in the immunostimulated male swiss albino mice.

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ABSTRACT

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation, initiated by free radicals (causing oxidative stress by reactive oxygen species - ROS). MDA is highly mutagenic and carcinogenic and plays a significant role in disease pathogenesis by disturbing the immune response and forms immune complexes that may be deposited in various tissues. This study is centralized on the immunostimulatory effect of Immunex DS (IDS) on the MDA level in abdominal muscle tissue of mice against Gene Vac B vaccine. Eight groups of male swiss albino mice (10 in each) were used in the present study, further categorised into 6 experimental groups A to F (IDS + vaccine), only IDS treated (group I) and control (group U) (untreated with IDS and vaccine) for comparison. A single dose of IDS @ 150mg/mouse is orally administered to all the groups of mice (except control group U) on day 0 of experiment. Six days (on day 7 of experiment) after IDS treatment (immunostimulation), varied doses of Gene Vac B vaccine is injected to all the 6 experimental groups of mice (A, 0.07ml/mouse; B, 0.1ml/mouse; C, 0.2ml/ mouse; D, 0.4ml/mouse; E, 0.8ml/ mouse and F, 1.0ml/mouse). Mice from all the eight groups were sacrificed three days after vaccine injection (on day 11, 12, 13, 14, and 15 of experiment). Abdominal muscle tissue was isolated and MDA activity was measured by kit method. It is interesting to note that the MDA activity in experimental groups correlates with the extent of tissue damage (due to lipid peroxidation) and cell necrosis during induced hepatitis B.
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Key words: MDA, Abdominal muscles, Immunostimulant, Hepatitis.

INTRODUCTION

Epidemiological and newly emerging infectious diseases threatening the life on earth, may be due to the lack of specific treatment or vaccine against infectious diseases. Immunostimulants are the non specific immune boosters that enhance the host immune system. They may be of natural/synthetic derived products of varied chemical composition, formulated against different modes and mechanisms of action. They stimulate the main factors of the immune system like phagocytosis, complement system, secretory IgA antibodies, α and Ɣ interferons, T and B lymphocytes, cytokines, pulmonary surfactant (Petrunov *et al*., 2007). They are used in adjuvant therapies in combination with vaccines conferring large scale applications in various fields of medical, pharmaceutical, life sciences, aquaculture, poultry and animal husbandry (Gelino *et al*., 2009). Immunex DS is a unique immunostimulant amalgamated with β carotenes, L-lysine, DL-methionine, essential fatty acids, Livamisol hydrochloride, vitamins (A, D_3, E, C and B_{12}), minerals (zinc, cobalt, manganese and selenium) and probiotics like *Lactobacillus* and *Saccharomyces cervisiae*. Hepatitis B is an infectious liver disease caused by hepatitis B virus (HBV). India is at a high prevalence (risk) rate accounting to about 4% with approximately 36 million carrier's worldwide (Tandon *et al*., 1996). HBV causes acute and chronic hepatitis and hepatocellular carcinoma (Uprichand *et al*.,

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2005). Progression from acute to chronic HBV infection is influenced by the patient age at acquisition of virus and it is observed that more than 90% of infected patients pertain lifelong infection (Lok and Mc Mahon, 2007; Hoofnagle *et al*., 2007). It is a world health problem, specifically in endemic regions such as Asia and Africa leading to hepatocellular carcinoma, liver decomposition, cirrhosis and premature mortality (Wang *et al*., 2015). Gene-Vac B is a non-infectious recombinant DNA Hepatitis B vaccine. It is a purified surface antigen (HbsAg) of virus obtained by culturing genetically engineered *Hansenula polymorpha* yeast cells. In 80s Esterbauer and his colleagues extensively studied MDA and many other aldehydes produced as secondary products of lipid peroxidation (Esterbauer *et al*., 1990, 1991; Poli *et al*., 1985; Benedetti *et al*., 1980; Cadenas *et al*., 1983; Winkler *et al*., 1984; Hurst *et al*., 1987 and Cheeseman *et al*., 1988). Lipid peroxidation is an oxidative conversion of polyunsaturated fatty acids to cytotoxic products like MDA (Bukan *et al*., 2003; Ayala *et al*., 2014). Oxidative stress is characterized by the increased production of free radicals and/or decreased activity of antioxidative defence (Burrin and Price, 1985). Imbalance between oxidant production and antioxidant defence may lead to many health problems (Hayes and Mc Lellan, 1993; Nakabeppu *et al*., 2004). Cancer and coronary heart diseases were reported due to the pathogenic effect caused by the intracellular production of ROS and oxidative stress (Gey, 1993; kunsch and Medford, 1999). In recent years oxidative damage caused due to pesticides, heavy metals and chemotherapeutic agent toxicities and the protection of mammalian cells against oxidative damage has become a prime aspect (Yousef *et al*., 2006; Prasenjit *et al*., 2008 and Desai *et al*., 2014). Oxidative stress is determined by the most reliable and popular marker like MDA in clinical situations and MDA is highly reactive and toxicity of this molecule is very relavent to biomedical research community (Giera *et al*., 2012). MDA is a low molecular weight aldehyde made up of 3 carbons and can be produced by different mechanisms (Rosenblum *et al*., 1989). It is a marker of cell membrane injury (Esterbauer *et al*., 1991). MDA production may be enzymatic/non enzymatic process and MDA is said to be more chemically stable and membrane permeable than ROS (Esterbauer *et al*., 1991) and it is believed that MDA originates under oxidative stress and is highly capable of reacting with various biomolecules like proteins and DNA leading to the formation of adducts resulting in biomolecular damage (Blair, 2008; Luczaj and Skrzydlewska, 2003). Excessive production of MDA is associated with different pathological states (Merendino *et al*., 2003; Slatter *et al*., 2004; Buskol *et al*., 2006; Negre-Salvayre *et al*., 2008; Shanmugan *et al*., 2008; Sanyal *et al*., 2009; Bartoli *et al*., 2011; Cheng *et al*., 2011; Li *et al*., 2012; Garcia *et al*., 2013 and Pizzimenti *et al*., 2013 and Zarkovic *et al*., 2013) and accumulated during ageing and chronic diseases. Phagocytic mobilization and activation may lead to tissue damage. MDA either directly act on biomolecules or indirectly activate the transcriptional factors involved in oxidation/reduction reactions (Alkalin *et al*., 2007). MDA plays a significant role in the evaluation of oxidative stress (Petievski *et al*., 2006) thereby acting as a direct marker of lipid peroxidation and indirect index of ROS activity (Gutteridge *et al*., 1990). Inspite of the presence of other biomarkers for measuring lipid peroxidation, MDA plays a significant role in measuring the oxidative damage caused due to oxidative stress and is utilized mostly during quantification in pathologies and toxicology associated with oxidative stress (Grotto *et al*., 2008). Hence, the present study is designed to assess the level of MDA which inturn interprets the cellular/ tissue damage caused due to oxidative stress due to IDS treatment and hepatitis B vaccine induction.

Material and Methods

Male *swiss albino* mice (*Mus musculus albinus*) (6-8 weeks old and 23 to 26 gm average weight) (eight groups) were used in the present study; they were fed with standard balanced diet *ad libitum* following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals). Six experimental groups A to F (10 in each group) and another group, I (10 mice) were administered orally with a single dose of IDS (@ 150mg/mouse) with the help of syringe fitted with a 3 inch 16 guaze oral, blunt feeding needle on day 0 of experiment. On day 7 of experiment (after 6 days of IDS treatment), all the experimental group mice A to F were injected with different doses of vaccine (A, 0.07 ml/ mouse; B, 0.1ml/mouse; C, 0.2ml/mouse; D, 0.4ml/mouse; E, 0.8ml/mouse and F, 1.0ml/mouse) and incubated for 3 days after vaccination. Ten mice of control group (U) (untreated with IDS and vaccine) was maintained for comparison. Two mice from all the 8 groups were sacrificed on day 1, 2, 3, 4 and 5 of experiment. The abdominal muscles were extracted and samples were assayed for MDA following the method of Armstrong and Browne (1994). Results were analysed for statistical significance using student's t test.

Results and Discussion

It is interesting to note that mice of group I showed an increased level of MDA when compared to controls (group U). MDA level in experimental mice of groups A to F was increased from day 1 to 5 when compared with group U and group I (except on day 1 and 2 in group A compared to group I). There was a

gradual rise in the level of MDA in all the experimental groups from day 1 to 5 (except in group C) and showed a peak response on day 5 of experiment (except in mice of group C on day 4). When MDA in the experimental groups of mice were compared among themselves, mice of group F showed the highest level of MDA with a peak level on day 5 of experiment (17.26 nanomoles of MDA/mg protein) (Table 1 and 2).

Table-1. Malondialdehyde (nanomoles of MDA/mg protein) content in the abdominal muscles of mice of different groups. Group A - treated with IDS @ 150 mg/mouse and vaccine @ 0.07 ml/mouse, Group B - treated with IDS @ 150 mg/mouse and vaccine @ 0.1 ml/mouse, Group C - treated with IDS @ 150 mg/mouse and vaccine @ 0.2 ml/mouse, Group I - IDS @ 150 mg/mouse and Group U (untreated with IDS and vaccine) mice during various days of experiment.

Day of sacrifice	Group A	Group B	Group C	Group I	Group U
	MDA	MDA	MDA	MDA	MDA
1	3.62	4.29	4.39	4.09	3.54
2	4.06	4.86	4.98	4.08	3.56
3	4.12	7.65	6.67	4.07	3.53
4	5.62	9.87	12.80	4.09	3.50
5	5.62	12.70	7.78	4.09	3.54

Values are expressed in the mean derived from 5 observations.

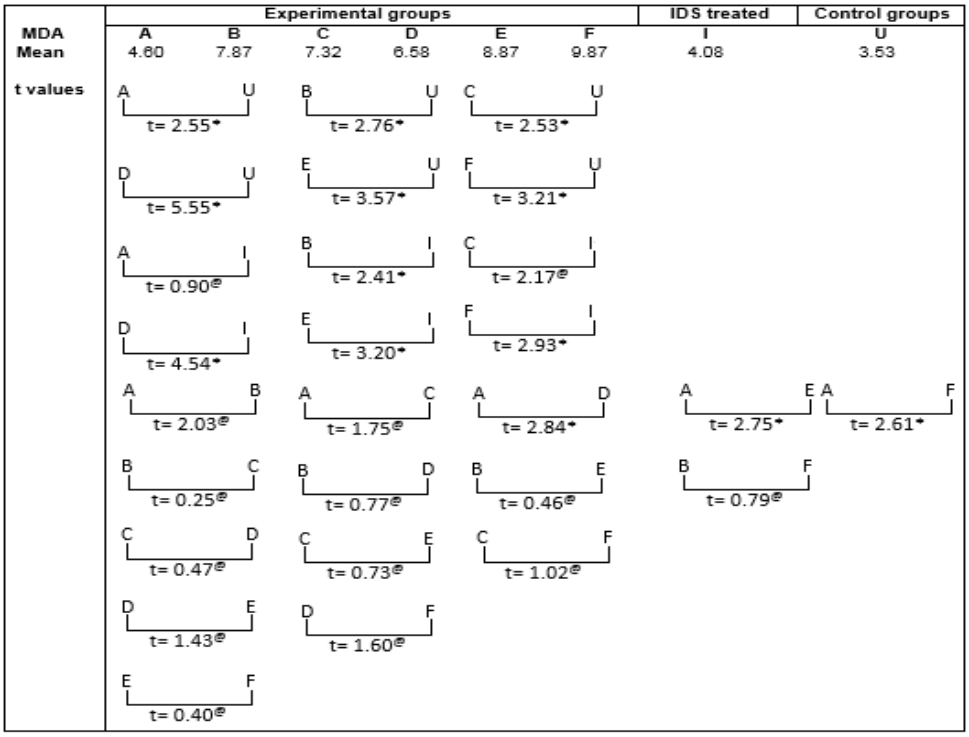
Table-2. Malondialdehyde (nanomoles of MDA/mg protein) content in the abdominal muscles of mice of different groups. Group D - treated with IDS @ 150 mg/mouse and vaccine @ 0.4 ml/mouse, Group E - treated with IDS @ 150 mg/mouse and vaccine @ 0.8 ml/mouse, Group F - treated with IDS @ 150 mg/mouse and vaccine @ 1.0 ml/mouse, Group I - IDS @ 150 mg/mouse and Group U (untreated with IDS and vaccine) mice during various days of experiment.

Day of sacrifice	Group D	Group E	Group F	Group I	Group U
	MDA	MDA	MDA	MDA	MDA
1	5.21	5.92	6.23	4.09	3.54
2	5.56	6.12	6.82	4.08	3.56
3	6.63	8.12	8.85	4.07	3.53
4	7.33	10.26	10.23	4.09	3.50
5	8.21	13.92	17.26	4.09	3.54

Values are expressed in the mean derived from 5 observations.

Significant increase in MDA was found in all the experimental groups (A to F) when compared with group U (controls) and I (IDS treated) (except in groups A and C) (Table 3). When the MDA level in experimental groups (A to F) were compared among themselves there was a non-significant difference (except the significant level of MDA in group A in comparison with D, E and F). Increased serum and

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



tissue MDA was observed in malignant tumors due to oxidative stress (Cirak *et al*., 2008). These results compare well with that of Saad and Ammar (2011) who also reported significant acceleration in the level of (lipid peroxide) MDA. Administration of Purslane (*Portulaca oleracea*) induced a significant reduction in the MDA levels in the liver and kidney of *albino* rats (Dkhil *et al*., 2011). Increased level of hepatic MDA was observed in isoniazid and rifampicin treated rats due to oxidation insult in the cell (Vishal *et al*., 2010). ROS cause significant increase of hepatic MDA level in mice fed with acrylamide and thymoquinone diet in cerebral cortex (Mehri *et al*., 2014). Lipid peroxidation was clearly reflected in increased MDA content in the kidney, liver and brain in response to NaF administration in mice (Sandeep *et al*., 2014).

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Conflict of Interests

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

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