Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
3(2):469-475
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

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.

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 and Tuddenham, 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 reminant 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 and Parvin, 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 sacrificied 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 (µg/100mg), RNA (µg/100mg) 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. The higher value of protein, DNA and RNA in IDS treated mice and the increase/decrease in the content of above constituents in IDS + vaccine treated mice compare well with that of Sakunthala et al., (2014) who also reported increase of stomach protein and DNA and much alteration in their level in IDS treated and IDS + vaccinated mice and that of Nathanael and Vardhani (2014) who found altered protein and DNA content in the liver of IDS + vaccine treated 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 and Viveka Vardhani (2014) Inoue et al., (2003) and 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	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
Mean	20.46	27.53	61.12	41.05	51.26	52.76	22.98	25.51

t values:

A – U: t = 1.32@	B – U: t = 3.47*	C – U: t = 2.86*		
D – U: t = 1.42@	E – U: t = 2.30*	F – U: t = 2.78*		
A – I: t = 2.70*	B – I: t = 1.92@	C – I: t = 2.70*		
D – I: t = 1.25@	E – I: t = 2.12@	F – I: t = 2.58*		
A – B: t = 3.06*	A – C: t = 3.01*	A – D: t = 1.59@	A – E: t = 2.46*	A – F: t = 2.95*
B – C: t = 2.51*	B – D: t = 1.06@	B – E: t = 1.92@	B – F: t = 2.34*	
C – D: t = 1.09@	C – E: t = 0.54@	C – F: t = 0.48@		
D – E: t = 0.57@	D – F: t = 0.71@			
E – F: t = 0.09@				

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	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
Mean	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	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
Mean	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.

References

1. Beckman, B. and Ames, B. 1997. Oxidative decay of DNA. J. Biol. Chem. 272: 19633-19636.
2. Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62: 315-323.
3. Ceriotti, G. 1955. Determination of nucleic acids in animal tissues. J. Biochem. 214: 59-70.
4. Cuthbertson, D. P. 1948. The significance of proteins in nutrition. Br. Med. J. 4581: 731-737.
5. Daniel, P. M. 1977. The metabolic homeostatic role of muscle and its function as a store of protein. Lancet, 2: 446-448.
6. Dukan, S and Nystronm, T. 1999. Oxidative and deterioration of growth arrested *Escherichia coli* cells. J. Biol. Chem. 274: 26027- 26032.
7. Fausto, N. 2000. Liver regeneration. J. Hepatol. 32: 19-31.
8. Feng-Min, L. and Hui, Z. 2009. Editorial: Management of hepatitis B in china. Chinese. Medical. J. 122(1): 3-4
9. Fyiad, A. A., Abd El-Kader, M. A. and Abd El-Haleem, A. H. 2012. Modulatory effects of pomegranate juice on nucleic acids alterations and oxidative stress in experimentally hepatitis rats. Life Science J. 9(3): 676-682.
10. Hertz, G. Z. and Stormo, G. D. 1999. Identifying DNA and protein patterns with statistically significant aligments of multiple sequences. Bioinformatics, 15(7/8): 563-577.
11. Inoue, M., Sato, E. F., Nishikawa, M., Park, A. M., Kira, Y., Imada, I. and Utsumi, K. 2003. Cross talk of nitric oxide, oxygen radicals and superoxide dismutase regulates the energy metabolism and cell death and determines the fates of aerobic life. Antioxidant. Redox Signal, 5(4): 475-484.
12. Islam, M. and Parvin, M. 2012. Antioxidant and hepatoprotective activity of an ethanol extract of Syzygium jambos (L.) leaves. Drug. Discov. Ther. 6(4): 205.
13. Jolles, P. and Werner, G.H. 1981. What's new in immunomodulation. Trends in Biochem. Sci. 6, 330-333.
14. Kasai, H. 1997. Analysis of a form of oxidative DNA damage, 8- hydroxy-2 deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat. Res. 387: 147-163.
15. Kehrer, J. P., Jones, D. P., Lemasters, J. J., Farber, J. L. and Jaeschke, H. 1990. Mechanisms of hypoxic cell injury: summary of the Symposium Presented at the 1990 Annual Meeting of the Society of Toxicology. Toxicol. Appl Pharmacol. 106(2): 165-178.
16. Kelly, D. A. and Tuddenham, E. G. 1986. Haemostatic problems in liver disease. Gut *27*: 339-349.
17. Liszewski, K. 2013. Deciphering RNA secondary structure. Genet. Eng. and Biotech. News. 33(18): 1-4.
18. Lowry, O. H., Rosenbrough, N. J. Farr, A. L. and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.
19. Malcolm, L. A. 1970. Growth retardation in a New Guinea boarding school and its response to supplementary feeding. Am. J. Clin. Nutr. 24: 297-305.
20. Michalopoulos, G. K. and DeFrances, M. C. 1997. Liver regeneration. Science, 276: 60-66.
21. Nathanael, P. J. R and Vardhani, V. V. 2014. The influenceof Immunex DS against experimental hepatitis B vaccine on liver protein and DNA profile of mice. Biolife 2(1):341-347.
22. Petrunov, B., Nenkov, P. and Shekerdjiisky, R. 2007. The role of immunostimulants in immunotherapy and immunoprophylaxis. Biotechnol and Biotechnol. EQ. 21(4): 454-458.

23. Rao, S. V., Einstein, J. W. and Das, K. 2014. Hepatoprotective and antioxidant activity of induced hepatotoxicity in rats. Intern. Letters. Natural Sciences, 3: 30-43.
24. Sakunthala, G., Nathanael, P. J. R and Vardhani, V. V. 2014. Impact of Immunostimulant on stomach protein and DNA activity during Hepatitis B infection in mice. Ind. J. Res. Tech. 2(2): 13-17.
25. Shih, C., Tai, P. C., Whitehead, W., Hosono, S., Lee, C. S. And Yang, C-S. 1996. Hepatitis B and C and liver cancer. In. J. R. Bertino (ed). Encyclopedia of cancer, vol II. Academic Press, Inc., Newyork, N.Y.
26. Sie, H. 1985. Oxidative Stress. Academic Press, New York.
27. Szabo, E., Lotz, G., Paska, C., Kiss, A., Scaff, Z. 2003. Viral hepatitis: New data on hepatitis C infection. Pathol. Oncol. Res. 9 (4): 215-221.
28. Tiollais, P.C., Pourcel, C. and Dejean, A. 1985. The hepatitis B virus. Nature. 317(6037): 489-495.
29. Tarakalakshmi, Y and Viveka Vardhani, V. 2014. Protein, DNA, RNA and amino acids contents from stomach of mice infected with Ancylostoma caninum larvae. Biolife. 2(2):486-492.
30. Ueda, J., Saito, H., Watanabe, H. and Evers, B. M. 2005. Novel and quantitative DNA dot-blotting method for assessment of *in vivo* *Lannea coromandelica* Linn. on thioacetami

proliferation. Am. J. Physiol. Gastrointest. Liver. Physiol. 288: G842-G847.
31. Vardhani, V.V. and Sakunthala, G. 2011. The specific role of liver in expelling *Ancylostoma caninum* larvae from the host system. The Bioscan. 6(2): 255-256.
32. Vardhani , V. V. and Sakunthala, G. 2012. Serum level of IgG and worm load in male swiss albino mice inoculated with L_3 larvae of *Ancylostoma caninum*. The Bioscan 7 (1): 65-67.
33. Verma, V. K. and Hussain, Z. 2013. Effect of 50% ethanolic extract of roots of *Cissampelos pareira* on some bio-chemical parameters to investigate the hepato-protective study in animal model (rats). J. Drug Discov. Therapeu. 1(7): 106-116.
34. Vinod Kumar, G. and Vardhani, V. V. 2013. Effect of ancylostomiasis on liver protein, aminoacids, and GST (Glutathine-S-transferase) level in male swiss albino mice. The Bioscan. 8(2): 459-462.
35. Zanetti, A. R., Van Damme P, Shouval, D. 2008. The global impact of vaccination against hepatitis B: a historical overview. Vaccine. 26 (49): 6266-6673.

DOI: https://dx.doi.org/10.5281/zenodo.7269589
Received: 3 April 2015;
Accepted; 11 May 2015;
Available online : 4 June 2015