BIOLOGIA (PAKISTAN)
June, 2018, 64 (1), 69-73

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Effect of Alloxan on IL-6 Gene Expression in *Mus musulus*

SYED SHAHID IMRAN BUKHARI[1]*, IQRA AZAM[1], MUDDASIR HASSAN ABBASI[1], MEERAB DAUD[1], NADEEM SHEIKH[2], ANDLEEB BATOOL[3], RABIA MAHMOOD[2] & MARYAM MUKHTAR[2]

[1]Department of Zoology Government College Of science Wahdat road, Lahore
[2] Department of Zoology University of The Punjab, Lahore
[3] Department of Zoology Government College University, Lahore

ABSTRACT

Alloxan monohydrate is an organic compound with heterocyclic skeleton; it has been the routine part of all bakery products such as pizza, biscuits and cakes etc. Swiss albino mice of six weeks were divided into control group and experimental group. Experimental group was administered with Alloxan monohydrate (200 mg/kg) intraperitoneally and then livers were extracted after 3, 6 and 12 hours. All the samples were processed for gel run and thermo cycler program, which revealed alterations in the expression of hepatic mRNA (IL-6) in all loads equally confirmed by the results of housekeeping gene β-actin. Gel analysis showed changes in the folds of IL-6 in hepatic mRNA compared to control. These finding led to the conclusion that Alloxan can induce an APR, which is evident by rise in expression of IL-6. Hence, extensive use of this food shiner should be avoided. Proposal of the current work was to determine the influence of Alloxan monohydrate on IL-6 gene expression, which is a major inflammatory marker.

Key words: Interleukin-6 (IL-6), Alloxan monohydrate, acute-phase response (APR).

INTRODUCTION

Maida or white wheat flour is used in many food stuffs worldwide. After removing fiber bran from the wheat flour maida is left behind. This is bleached with alloxan for more shininess and whitening. Alloxan is utilized in food items such as maida flour and in other bakery products (Sudha *et al*., 2014) but alloxan is toxic to Pancreas (Rahiman, 2012). It is being incorporated in human bodies through white bread, pizza dough, cookies and cakes, or products containing white flour (veracity, 2005).

The acute-phase response (APR) being a very complex reaction, affect the host with infection, inflammation and trauma (Jain *et al*., 2011). It is imputed to class of metabolic and systemic alterations occurring within hours of stimuli of inflammation thus enclosing a large variety of pathological effects e.g. such as leukocytosis, pyrexia, hormone variations, and depletion of muscle protein. These all combine to reduce the tissue breakdown along with boosting the system to repair damage (Martinez and Ceron, 2005). APR mirror all the changes occurring in organism immediately after tissue damage in order to reinstate itself to the position of homeostasis (Gould and Dyer, 2010). After APR, acute phase proteins (APPs) change the concentration of plasma by negative or positive acute phase (Ceciliani *et al*., 2002). APPs are the class of proteins, whose concentrations affect changes in hepatocytes. The immensity of APPs increases about 50% in the case of serum amyloid A (SAA) and C-reactive protein (CRP) (Bode *et al*., 2012).

Positive APPs are categorized further as minor, moderate and major, depending on the level of magnitude. Positive acute phase proteins are the part natural immune system and also have physiological functionality difference. Some of them act to shatter or reduce the microbial growth, e.g., C-reactive protein, mannose-binding protein, complementary substituent, ferritin, ceruloplasmin, SAA and haptoglobin respond negatively to inflammation response (Herpers *et al*., 2009). Negative acute phase proteins start to diminish in concentration by more than 25% in plasma during

Author's Contribution: S.S.I.B., Designed and supervised the project; **I.A.,** Executed the experiment; **M.D.,** Performed the experiment; **M.M. & R.M,** Provided technical help; **A.B.,** Helped in literature review, results and discussion; **M.H.A.,** Supervised the project; **N.S.,** Provided lab facility and helped technical write up
***Corresponding author**: shahidimran1332@gmail.com

inflammatory response. This decrease can happen fastly (within 24 hours) or may decrease slowly within a couple of days. Two principles, negative acute phase proteins are transferrin and albumin (Peterson *et al*., 2004).

Functionally immune system depends largely on interleukins, which are family of cytokines (excreted proteins and signal molecules) being expressed by white blood cells e.g., leukocytes (Brocker *et al*., 2010). Fifteen various peptides with low molecular weight mediators are produced by activated leukocytes (interleukins) and other cells. These are in large named cytokines and are indulged in ameliorating acute phase responses.

Interleukin-6 (IL-6) is the main mediator for hepatocytes secretion of most of the APPs. IL-6 was distinguished as a T-cell-derived B-cell differentiation factor. It prompted B cells to discriminate into antibody-producing cells. IL-6 is secreted by different types of cell, such as fibroblasts, keratinocytes, T cells, B cells, monocytes, endothelial cells, meningeal cells, and some tumor cells (Banks *et al*., 1994). IL-6 also designated as beta cell stimulating factor-2 (BSF-2) and interferon Beta-2, is chemically from cytokine family is involved in a wide range of functions occurring in the biological systems (Hirano *et al*., 1986). It is a prime mediator of pyrexia and acute phase response of crossing the blood-brain barrier (Munoz *et al*., 2013).

Alloxan is used in our food such as Maida flour and in bakery product Maida flour is prepared after removing rich bran from wheat (Sdha, *et al*., 2014). Then this white flour is bleached with Alloxan to make it smooth, fine and white. Alloxan is toxic to Pancreas in human body being the complementary part of maida or white wheat flour is used in many food stuffs worldwide (Rahiman, 2012). Alloxan can be incorporated in humans it can be found in white bread, pizza dough, cookies and cakes, or added to white the flour. Instead, it is a by-product of the flour bleaching process when the chlorine reacts with protein of the flour (Francois, 2015). It originates diabetes since it spins up huge amounts of radicals in the beta cells of pancreas, thus damaging them (Mercola, 2009). The uric acid and its derivatives start the free radical harm to DNA in the beta cells thus causing their malfunctioning and death. After the damage of these beta cells there is failure of their normal operation thus reducing the production of insulin (Veracity, 2005). Alloxan-mediated diabetes enhances the ultra-structural changes in liver morphology, ranging from steatosis to Alloxan decompose into alloxanic acid in the absence of reluctant. Whereas in glutathione presence, it is converted into dialuric acid through alloxan radical, which immediately oxidize automatically back to alloxan. During this redox cycling mechanism, ROS (reactive oxygen species) are produced thus destroying beta cells in Langerhans Islets. Water soluble Alloxan can produce acute phase response (Milne *et al*., 2005). Alloxan is an organic compound based on a pyrimidine heterocyclic skeleton. It is a toxic analog of glucose having high affinity for water and therefore exists as the monohydrate (Rohilla and Ali, 2012).

In view of the aforementioned literature cited, a number of experimental studies have been printed on the effects of Alloxan exposure in human patients and experimental organisms. To the best of our knowledge no or relatively very little is known concerning changes in IL-6 gene expression in Alloxan induced animal model. Therefore, current work was undertaken to find the variation in gene expression in male albino mice.

MATERIALS AND METHODS

Analytical grade chemicals were used and bought through commercial sources: Trizol reagent was used for RNA isolation from cells; real-time polymerase chain reaction (PCR) primers. Homogenizer (HG-15D) Germany; Micro centrifuge (SIGMA2-16PK); Germany Nanodrop (OPTIZEN-Nano Q); UV-Vis (Spectrophotometer) Photodoc Imaging system (USA); Thermo cycler (Multi Gene-OPTIMAX); TRI zoln Genei^{TM} (Lot No. 643051); Germany Wiz Script^{TM} (Korera) were used during the experimentation. All chemicals and reagents were purchased from sigma (Germany).

Experimental Design

Male Swiss albino mice of about 30±5 grams were divided randomly divided into two distinct groups, experimental and control group. Experimental group was further subdivided in four groups on the basis of different time slots 3, 6, 12 and 24 hours. All animals were reared under the standardized conditions of 12-hours of light/dark and were provided with fresh water and food *ad libitum*. Experimental groups were subjected to intraperitoneal injection of 0.1ml of 200mg/kg Alloxan and sacrificed after the respective designated time points. Livers were excised, rinsed with saline and stored at -20 °C till further study.

Total RNA Extraction and cDNA synthesis

Liver tissue sample (100mg) was taken in an eppendrof tube and total RNA was extracted with 1ml Trizol reagent as per manufacturer's manual instructions. RNA suspension was stored at -20°C. Purity and quantity of RNA was evaluated by Nano drop Spectrophotometer (UV/VIS). cDNA was produced by reverse transcription of isolated RNA using Wiz Script ^{TM} RTase (200U/ µl) kit. Gradient PCR was performed for Primer optimization with the following ingredients and volume was added to make total reaction of 25µl.

Primers sequence

IL-6 gene expression was assessed using PCR. β-actin (endogenous control) during experiment for equal loading of sample.

Agarose gel electrophoresis

2% resolving gel was made for DNA separation and gel was captured using Gel Doc-it^{TM} imaging system. Gel was analyzed using image J software to find the level of expression of mRNA of IL-6.

RESULTS

RNA and cDNA were measured and noted (Table). Group III showed maximum concentration of RNA with 2335.7 (ng/µl) and cDNA showed concentration of 173.1(ng/µl). Group II showed minimum concentration of RNA with 1195.4 (ng/µl) and cDNA showed concentration of 287.7 (ng/µl). Group I showed minimum concentration of RNA with 1263.9 (ng/µl) and cDNA showed concentration 196.6(ng/µl). Group IV showed minimum concentration of RNA with 1049.4(ng/µl) and cDNA showed concentration of 214.0(ng/µl).

Table I: RNA and cDNA quantification

Groups	RNA (ng/µl)	Absorbance A_{260}/A_{280}	cDNA (ng/µl)	Absorbance A_{260}/A_{280}
Control group	116.4	1.83	194.9	1.57
Group I (3 hrs)	1263.9	1.84	196.6	1.51
Group II (6 hrs)	1195.4	1.84	287.7	1.66
Group III (12 hrs)	2335.7	1.98	173.1	1.80
Group IV (24 hrs)	1049.4	1.92	214.0	1.62

Expression of hepatic mRNA's

Gel study of PCR products showed the considerable changes in the gene expression of hepatic mRNA IL-6 in all experimental groups in comparison to control. Equal loading of the samples was confirmed by the results of the house keeping gene β-actin. The expression shown here reflected almost bands of around 100 bps. Entire experimental group showed clear bands.

DISCUSSION

In the vertebrate's immune system foreign pathogens is commonly accepted to function as defense system of host. IL-6, an important mediator of inflammation is also a signal activator for signal transduction release and different survival factors such as Pleiotropic cytokine IL-6. These factors play role during the inflammatory process to block apoptosis in cells thus keeping cells alive into toxic surroundings. As Interleukin-6 (IL-6) play both as anti-inflammatory myokine and pro-inflammatory cytokine thus becoming the activator of the most acute phase proteins.

The current study was undertaken to find the changes in the IL-6 gene expression with the administration of Alloxan in mice up to 24 hours compared to control group. IL-6 gene, act as pro-inflammatory cytokine, whose

expression increases during inflammation or other tissues. IL-6 evokes the transcription of different types of hepatic genes during the acute phase response, having many genes involved in metabolic process, transcriptional regulation and synthesis of proteins.

In present study expression of IL-6 was noted after Alloxan induction in all the time point under study. This might be due to the reason that IL-6 is +ve acute phase protein and IL-6 signaling is one of the main acute-phase response regulator in the hepatocytes (Heinrich *et al*., 2003) reported about inflammatory stimulus, during which IL-6 was excreted and attached to a IL-6 receptor α and gp130 complex. The IL-6 ligand-receptor interaction caused the mediation of Janus kinases (JAKs), that phosphorylated signal transducers and activators of transcription (STAT) proteins, predominantly STAT3 (Nagashima *et al*., 2004). Upon phosphorylation at tyrosine residue 705, STAT3 translocates into the nucleus, where it causes the regulation in the transcription of many targeted genes (Levi *et al*., 2002). Similarly, findings of up regulation of IL-6 have been observed in Turpetine Oil induced rat model to stimulate acute phase response (Sheikh *et al*., 2007). Abbasi *et al*., (2014) reported an early rise of IL-6, gene expression in *Nerium Oleander* leaves induced APR, in rats.

In a present study, it was observed that as the Alloxan was injected to each mice of experimental group and Alloxan level increased in all animals, while the control group did not received any treatment. Alloxan effects were observed on liver's IL-6 gene. Alloxan effects IL-6 in such a way that it changes the folds of hepatic mRNA.

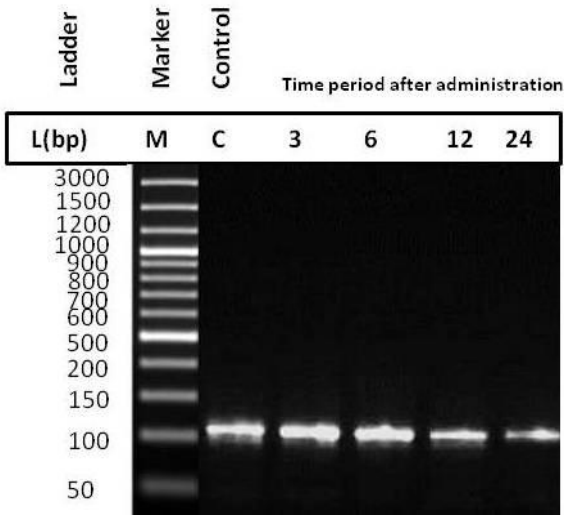

Fig 1: Gel analysis

Gel analysis via Image illustrated significant expression of mRNA's in the experimental groups as compared to control group.

CONCLUSION

Conclusively stated that very extensive usage of Alloxan in foods cause very serious health problems as observed or revealed by variation in IL-6 gene in liver. Furthermore, it is also found that the continuous use of this food additive may induce serious harm to human health.

REFERENCES

Abbasi, M. H., Fatima, S. & Sheikh, N., 2014. Histological comparison of natural lung injury in *Rattus norvegicus* induced by a natural herb (*Nerium oleander*) and a known carcinogen (thio-acetamide). *Bilogia (Pakistan).*, **61** (2): 135-138.

Banks, W. A., Kastin, A. J. & Gutierrez, E.G., 1994. Penetration of interleukin-6 across the murine blood-brain barrier. *Neurosci. Let.*, **179(1)**: 53-56.

Bode, J. G., Albrecht, U., Haussinger, D., Heinrich, P. C. & Schaper, F., 2012. Hepatic acute phase proteins–regulation by IL-6 and IL-1 type cytokines involving STAT3 and its crosstalk with NF-κ B-dependent signaling. *European J. Cell Boil.*, **91(6)**: 496-505.

Brocker, C., Thompson, D., Matsumoto, A., Nebert, D.W. & Vasiliou, V., 2010. Evolutionary divergence and functions of the human interleukin (IL) gene family. *Human Genomics*, **5(1)**: 30.

Ceciliani, F., Giordano, A. and Spagnolo, V., 2002. The systemic reaction during inflammation: the acute-phase proteins. *Pro. Pep. Let.*, **9(3)**: 211-223.

Gould, B. E. & Dyer, R., 2010. Pathophysiology for the Health Professions-E-Book. Elsevier Health Sciences. 2nd ed Willy sons. New York 1248 pp.

Heinrich, P. C., Behrmann, I., Serge, H., Hermanns, H.M., Muller-Newen, G. and

Schaper, F., 2003. Principles of interleukin (IL)-6 type cytokine signaling and its regulation. *Biochem. J*., **374(1)**: 1-20.

Herpers, B. L., Vlaminckx, B., Burkhardt, O., Blom, H., Biemond-Moeniralam, H.S., Hornef, M., Welte, T. & Kuijper, E.J., 2009. Intravenous tigecycline as adjunctive or alternative therapy for severe refractory Clostridium difficile infection. *Clin. Infec. dis*., **48(12)**: 1732-1735.

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S.I., Nakajima, K., Koyama, K., Iwamatsu, A. & Tsunasawa, S., 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature*., **324 (6092)**: 73-76.

Jain, S., Gautam, V. & Naseem, S., 2011. Acute-phase proteins: As diagnostic tool. *J. Pharm. Bio. Allied Sci.*, **3(1)**: 118.

Levi, D.M., Hariharan, S. & Klein, S.A., 2002. Suppressive and facilitatory spatial interactions in peripheral vision: Peripheral crowding is neither size invariant nor simple contrast masking. *J. Vision*., ***2*(2)**: 3-3.

Martinez-Subiela, S. & Ceron, J.J., 2005. Evaluation of acute phase protein indexes in dogs with leishmaniasis at diagnosis, during and after short-term treatment. *Veterinarni Medicina*. **50:** 39-46.

Milne, G. W. ed., 2005. Gardner's commercially important chemicals: synonyms, trade names, and properties. 2nd ed. John Wiley and Sons. 1130.

Munoz-Canoves, P., Scheele, C., Pedersen, B. K. & Serrano, A.L., 2013. Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? *F.E.B.S. J.,* **280**(**17**): 4131-4148.

Nagashima, A. & Arzt, E., 2004. Intracellular proteins and mechanisms involved in the control. *J. Clin. Invest.*, **109(9)**: 1143.

Petersen, H. H., Nielsen, J.P. & Heegaard, P.M. H., 2004. Application of acute phase protein. *Biochem. J.*, **34(5)**: 10-30.

Rohilla, A. & Ali, S., 2012. Alloxan induced diabetes: mechanisms and effects. *Inter. J. Res. Pharmaceut. Biomed. Sci.,* **3(2)**: 819-823.

Sudha, M. L., Rajeswari, G. & Rao, G.V., 2014. Chemical composition, rheological, quality characteristics and storage stability of buns enriched with coriander and curry leaves. *J. Food Sci. Tech.*, **51(12)**: 3785-3793.

Veracity, D., 2005. White flour contains diabetes-causing contaminant alloxan. *Natural News. Com.*