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QUERCETIN MODULATES Na^+/K^+ ATPASE AND SODIUM HYDROGEN EXCHANGER IN TYPE 2 DIABETIC ERYTHROCYTES

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Abstract

Quercetin has been reported to possess many important biological properties. We undertook *in vitro* studies to examine the effect of quercetin on erythrocyte Na+/K+ ATPase and sodium hydrogen exchanger (NHE) activity in normal and type 2 diabetic subjects. A significant ($p<0.02$) increase in the activities of Na+/K+ ATPase and a decrease ($p< 0.01$) in NHE were observed in type 2 diabetic subjects compared to normal. *In vitro* treatment with quercetin caused inhibition of both Na+/K + ATPase and NHE. The inhibitory effect of quercetin was concentration dependent. The effect of quercetin on Na+/K+ ATPase and NHE may be explained due to a direct effect of this compound on plasma membrane leading to a change in membrane fluidity. The data obtained in this study may help to explain the anti-diabetic and anti-carcinogenic role of quercetin.

Key words: Diabetes, erythrocytes, quercetin, Na+/K+ ATPase, NHE.

Article information

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INTRODUCTION

Genetic susceptibility to type 2 diabetes coupled with lifestyle changes is a major cause for the growing incidence of diabetes in Asian countries (27). Long term consequences of uncontrolled diabetes are serious and result in diabetic neuropathy, nephropathy, retinopathy, amputations, and cardiac dysfunction. Significantly, in most cases these alterations are irreversible thus prevention of these late complications assumes great importance. Alterations in erythrocytes both at the membrane and intracellular level have been used as markers to assess the development of long term vascular complications of diabetes (36, 33). Changes in erythrocyte membrane bound Na+/K+-ATPase (31,7), Na/H antiport (22, 2), rheological properties (37) and intracellular redox parameters (25, 31, 28) have been reported. Frequently these alterations have been correlated with the development of several late complications of diabetes.

Sodium pump or Na+/K+ ATPase is an ubiquitous membrane-associated protein complex that is expressed in most eukaryotic cells. The pump transduces energy from the intracellular hydrolysis of ATP to achieve the counter transport of sodium and potassium across the cell membrane (35). It is widely believed that impairment in Na^+/K^+ ATPase activity may play a major role at the cellular level in the pathophysiology of many late complications of diabetes mellitus (11) and in the development of diabetic vascular complications (13). The Na^+-H^+ antiport (NHE) catalyzes amiloride sensitive exchange of Na^+ for H^+. This transport exists in virtually all cell types and plays a major role in pH homeostasis, cell volume regulation and cell proliferation. NHE is one of the most studied plasma membrane mechanisms involved in proton transport (8) and directly controls essential parameters such as cellular pH, volume and growth. Altered NHE activity has been linked to the pathogenesis of several diseases including essential hypertension, diabetes, congenital secretory diarrhea, tissue damage caused by ischemia/reperfusion, and oncogenic transformation (24).

Flavonoids are a group of naturally occurring, low molecular weight benzo-γ-pyrone derivatives, ubiquitous in plants. Quercetin (3,3',4',5',7-pentahydroxylflavone) (Figure. 1) a well known antioxidant of flavonoid group and has recently received much attention due to several reported health benefits (1). The mechanism(s) contributing to the health beneficial effects of quercetin involve the quenching of free radicals, elevating antioxidant status, and membrane modulating effects (29, 30).

Previously we have shown the effects of green tea catechins on erythrocyte membrane transport systems in type 2 diabetic patients (31). The present study was undertaken to study the effect of quercetin on erythrocyte membrane Na+/K+-ATPase and Na/H antiport in type 2 diabetic patients.

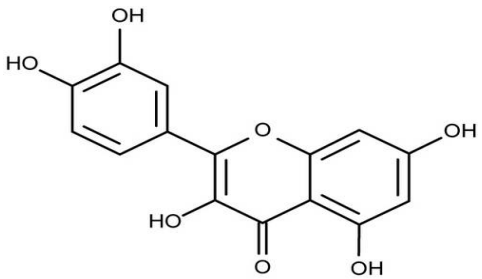

Figure 1. Structure of Quercetin.

MATERIALS AND METHODS

Selection of subjects

Human volunteers (n = 31) of sound health in the age group of 56 ± 8 years and having fasting blood glucose level 85 ± 14 mg/dl with no evidence of any clinical abnormality were used as control. The BMI of these subjects was in the range of 24.8 ± 3.8 kg/ m^2. None of the controls had family history of diabetes mellitus or hypertension from last two generations. Likewise (n = 31) type 2 diabetic patients were selected on the basis of previously reported

criteria (32). Diabetic patients were in the mean age group of 58 ± 7 years with fasting blood glucose of 183 ± 42 mg/dl and BMI 27 ± 4 Kg/m^2. Mean total plasma cholesterol was recorded 209 ±50 mg/dl and the duration of diabetes was 12 ± 5 years. None of the patients had high blood pressure or microalbuminuria. Care was also taken to exclude patients who had a family history of hypertension. Fasting blood was taken both from control and diabetic subjects after informed consent.

Blood collection and isolation of erythrocyte membrane

Using ACD as anticoagulant, venous blood was collected both from control and type 2 diabetic patients after an over night fast. The blood samples were stored at 4º C and were processed within four hours of collection. The blood samples were centrifuged at 4 ºC for 10 minutes at 1000 x g to remove plasma and buffy coat to obtain packed erythrocytes. The isolated erythrocytes were washed 4-5 times with 0.154 M NaCl. The method proposed by Marchesi and Palade (20) was followed to obtain erythrocyte membrane from leukocyte free red cells.

Determination of membrane Na^+/K^+ - ATPase activity

Ouabain-sensitive Na^+/K^+-ATPase activity was determined following the method of Suhail and Rizvi (34). The final assay mixture comprised 140 mmol/l NaCl, 20 mmol/l KCl, 3 mmol/l $MgCl_2$, 30 mmol/l imidazole (pH 7.25) ± 5 x 10^{-4} mol/l ouabain, 6 mmol/l ATP and contained 0.4–0.9 mg /ml protein. The assay mixture was incubated for 30 min at 37°C. Thereafter the reaction was stopped by the addition of a solution containing 0.5 mol/l H_2SO_4, 0.5% ammonium molybdate and 2% SDS. The amount of inorganic phosphate liberated was estimated by the method of Fiske et al. (6). In vitro experiments were carried out by adding quercetin to the enzyme assay medium and incubating at 37°C for 30 min. prior to enzyme assay. In parallel control experiments the assay medium was incubated without quercetin.

Estimation of NHE activity

NHE activity was estimated in isolated erythrocytes in terms of amiloride-sensitive H+-efflux from acid loaded cells as per the previous reports (22). The activity of the antiport was evaluated by the difference in hydrogen efflux rates from acid loaded erythrocytes in the presence and absence of the inhibitor amiloride. Suspension was made by adding 0.2 ml of packed erythrocytes into a solution of 3.8 ml containing 150 mmol/l NaCl, 1 mmol/l KCl, 1 mmol/l $MgCl_2$, 10 mmol/l glucose. The cell suspension was incubated at 37°C for 5 min using magnetic stirrer. pH of the cell suspension was brought to pH 6.35–6.45 within 10 minutes using 0.2 mol/l HCl solution in 150 mmol/l NaCl. DIDS (4,4-diisothiocyanatostilbene-2,2-disulfonic acid) was added then (0.2 mmol/l final concentration) and the pH of the medium was brought to 7.95–8.00 using 0.05 mol/l NaOH solution in 150 mmol/l NaCl. In a parallel running experiment, amiloride (0.5 mmol/l final concentration) was added with DIDS. The first minute proton efflux was registered thereafter. The rate of NHE, expressed in µmol/l of cells/hr. was derived from the difference in rates of medium acidification in the absence (ΔpH_1) and presence (ΔpH_2) of amiloride, corrected by the buffer capacity of the incubation medium (b), the cell volume in the suspension and the incubation time.

In vitro experiments were carried out by adding quercetin to the cell suspension and incubating for 30 min at 37°C prior to antiport assay. In parallel experiments, control cells were incubated in the assay medium with the solvent but without quercetin. Hemolysis in erythrocytes was assessed by measurement of the hemoglobin released from cells, relative to the total cellular content. After incubation the samples were centrifuged at 2000 x g and the hemoglobin concentration of supernatants was determined spectrophotometrically.

Other biochemical measurements

Accu Check Glucometer (Roche) was used to detect blood glucose values of samples. Erythrocyte membrane protein was estimated following the method of Lowry et al. (19). Statistical analysis of the data was performed employing ANOVA followed by Student's t test, with a $p<0.05$ considered significant. Prism 5.0 was the statistical software used in this study.

RESULTS AND DISCUSSION

We observe a significant decrease in the activity of Na^+/K^+ ATPase in diabetic subjects as compared to normal healthy controls ($p<0.02$) (Table 1). Our findings are in agreement with previous studies (33, 16). Erythrocyte

Table 1. Erythrocyte membrane Na+/K+ ATPase and NHE activity in normal healthy subjects and type 2 diabetic patients.

Subjects	Normal healthy controls	Type 2 diabetic patients
Number of subjects (n)	31	31
Age (Years)	56 ± 8	58 ± 7
B.M.I. (Kg/m^2)	24.8 ± 3.8	27 ± 4
(Na^+-K^+)-ATPase activity	0.0433 ± 0.0060	0.0323 ± 0.0068 *
Sodium-hydrogen exchanger (NHE) activity	5042.484 ± 753.6378	6776.257 ± 1063.4230 **

Na^+/K^+-ATPase activity (ouabain-sensitive) is expressed in terms of µmol pi released/hr./mg membrane protein at 37°C. NHE activity expressed as proton efflux µmol/l erythrocyte/hr. at 37°C. Values represent means±S.D.

* Values are statistically significant at $p<0.02$, with respect to healthy controls.

** Values are statistically significant at $p<0.01$, with respect to healthy controls.

Na^+/K^+-ATPase is believed to play a pivotal role in the regulation of intra- and extracellular cation homeostasis. The decrease in the activity of Na^+/K^+-ATPase in diabetic condition may be due to altered functional and compositional changes in membrane during diabetic condition. A correlation between altered Na^+/K^+-ATPase activity and membrane fluidity has been reported by Mazzanti et al. (23).

Due to the presence of higher glucose concentration in diabetic condition, various membrane proteins get glycosylated, thus glycosylation may be one of the causes for the decrease in the activity of Na+/K+ ATPase. A decrease in the activity of Na^+/K^+-ATPase has also been observed in alloxan diabetic rat erythrocytes, kidney, brain (15), diabetic lens and glomerulus (4).

Incubation with quercetin caused a concentration-dependent inhibition of Na+/K+ ATPase activity in both normal and type 2 diabetic erythrocytes (Figure 2). The inhibitory effect of quercetin was significant ($p< 0.01$) in micro molar range in both normal and type 2 diabetic erythrocytes. Quercetin thus acts as Na+/K+ ATPase inhibitor at micromolar concentration. This finding may be useful in conditions where enhanced activity of Na+/K+ ATPase has been observed (10).

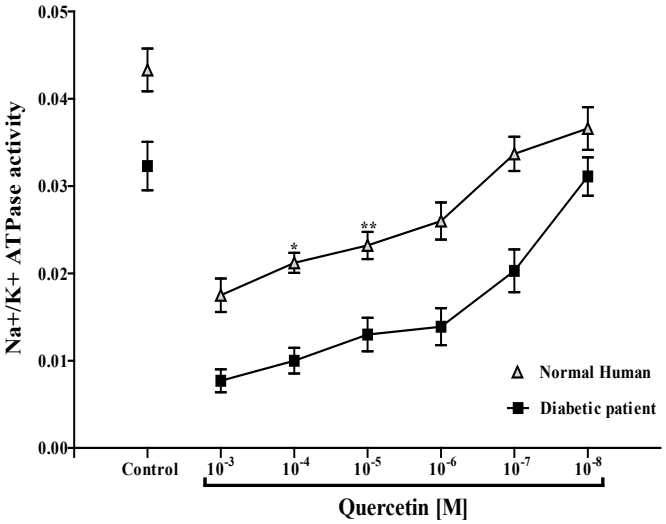

Figure 2. Concentration dependent effect of quercetin on erythrocyte membrane Na/K -ATPase activity in normal humans and diabetic patients. Na/K -ATPase activity expressed in terms of micromol pi released / hr./ mg membrane protein at 37° C. Values are means ± S.D.

Studies have already shown that a number of dietary components may influence membrane characteristics such as fluidity, stability and susceptibility to membrane oxidative damage (26). The role of oxidative damage of membrane in NIDDM may also contribute to the alteration in activities of membrane bound enzymes. Oxidative damage has been implicated in the cellular dysfunction and complications of diabetes (12). Due to increased oxidative stress in diabetes, conformational changes in Na^+/K^+ATPase is observed which results in decreased Na^+/K^+ATPase activity.

A significantly ($p<0.01$) elevated activity of erythrocyte NHE was observed in type 2 diabetic subjects as compared to normal controls. Table no. 1 shows the activity of sodium-hydrogen exchanger in both normal and type 2 diabetic erythrocytes. Results obtained in this study are consistent with the results of Matteucci et al. (21) and Rizvi and Zaid (31) who observed that diabetic patients, both normo and micro albuminuric, had a Na^+/H^+ antiport activity significantly higher than control subjects. Abnormal activities of NHE have been reported in essential hypertension and type 1 insulin dependent diabetes mellitus (IDDM). Likewise elevated NHE activity has been observed in lymphocytes of patients affected by obesity and type 2 diabetes mellitus (9).

A decreased response of Na^+-H^+ antiport to insulin and leptin has been observed in erythrocytes from obese individuals in comparison to normal erythrocytes (14). According to Matteucci (21), a linear and significant correlation is found between Na^+/H^+ exchange and some cardiac indexes suggesting that increased Na^+/H^+ antiport activity may be a possible predictive risk factor for the development of diabetic cardiomyopathy. Matteucci and Giampietro (21) observed a relationship between Na^+/H^+ exchange and microalbuminuria in IDDM patients and found that Na^+/H^+ exchange was elevated to a similar extent in diabetic patients with and without microalbuminuria.

In our study quercetin was observed to be potent inhibitor of NHE. Pretreatment of erythrocytes with quercetin blocked NHE activity in both normal and type 2 diabetic subjects at micromolar concentrations ($p<0.05$). (Figure 3). The biochemical mechanisms responsible for the flavonols induced inhibition of NHE activity, may involve protein kinase dependent or independent pathways. The phenolic groups of flavonoids are supposed to react with extracellular domains of NHE (22). Evidence shows that changes in cytosolic pH caused by the activity of transporters such as NHE may be part of a mitotic signalling pathway (5). Activation of NHE is an early event in the response of cells to mitogenic growth factors. It is known that NHE plays an important part in oncogenic transformation. Stimulation of NHE and the subsequent intracellular alkalinization seem also to participate in the regulation of programmed cell death (apoptosis) (18). Thus the strong inhibitory effect of quercetin on NHE assumes great importance.

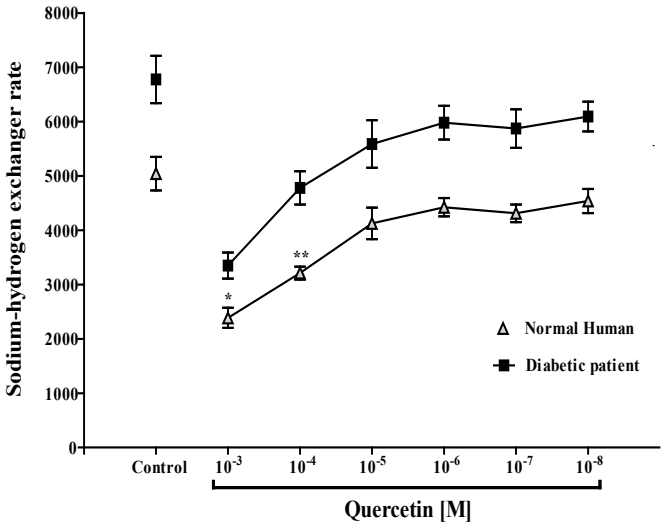

Figure 3. Concentration-dependent effect of quercetin on erythrocyte NHE activity in normal humans and diabetic patients. NHE activity expressed as proton efflux µmol/l erythrocyte/h at 37°C. Values are means ± S.D.

An altered erythrocyte Na+/K+ ATPase and NHE in type 2 diabetic patients may lead to deranged cell volume regu-

lation thereby contributing to the pathophysiology of several disorders such as liver insufficiency, diabetic ketoacidosis, hypercatabolism, fibrosing disease, sickle cell anemia, and infection (17). The role of insulin in modulating intracellular ion homeostasis with the concerted action of Na+/K+ ATPase and NHE has been reviewed (10), it has been hypothesized that transduction of a hormonal signal primarily involves alteration of membrane ion transport followed by a change in cell volume. This change in cell volume may assist in executing a hormonal stimulus on cell function. Quercetin produces beneficial effects for the human health via scavenging of free reactive oxygen species, metal chelation and stimulation, inhibition of enzyme activities, and signal transduction pathways. Ultrastructural observation reveals changes in membranous structures, after flavonol treatment (3). Based on the effect of quercetin on erythrocyte Na+/K+ ATPase and Na/H antiport in diabetic patients, we conclude that quercetin exerts a potent affect at the membrane level modulating the activity of membrane transport processes.

Other articles in this theme issue include references (39-66).

REFERENCES

1. Alexandra B. B., A Review of Quercetin: Chemistry, Antioxidant Properties, and Bioavailability. *Journal of Young Investigators.* 2009, **19**(10).

2. Bourikas, D., Kaloyianni, M., Bougoulia, M., Zolota, Z., Koliakos, G., Modulation of the Na^+-H^+ antiport activity by adrenaline on erythrocytes from normal and obese individuals. *Molecular and Cellular Endocrinology*. 2003, **205:** 141-150.

3. Bożena P. P., Wiesław I. G., Lucjan M., Roman P., Tomasz P., Barbara Z., Jarosław P., Antoni G., *Biochimica et Biophysica Acta.* 2007, **1768**: 2195–2204.

4. Cohen, M.P. Aldose reductase, glomerular metabolism, and diabetic nephropathy. *Metabolism.* 1986, **35(1)**: 55-9.

5. Counillon, L., Pouyssegur J., Structure function studies and molecular regulation of the growth factor activable sodiumhydrogen exchanger (NHE-1). *Cardiovasc Res*.1995, **29**:147– 154.

6. Fiske, C., Subbarow, Y., The colourimetric determination of phosphorus. *J Biol Chem.* 1925, **66**: 375– 400.

7. Gallicchio, M. A., Bach, L. A., Advanced glycation end products inhibit Na^+ K^+ ATPase in proximal tubule epithelial cells: role of cytosolic phospholipase A2alpha and phosphatidylinositol 4-phosphate 5-kinase gamma. *Biochim Biophys Acta*. 2010 Aug, **1803(8)**: 919-30.

8. Ganapathy V, Leibach FH., Proton and regulation of biological functions. *Kidney Int* 1995; **40** :S4–S10.

9. Ghigo**,** D., Alessio, P., Burzacca, S., Costamagna, C., Anfossi, G., Cavalot, F., Bosia, A., Trovati, M., Na^+/H^+ antiporter activity in peripheral blood lymphocytes of obese and type 2 diabetic patients is increased only in the presence of arterial hypertension. *Eur J Clin Invest*. 1994 Mar, **24(3)**: 166-72.

10. Graf, J. and Häussinger, D., Ion transport in hepatocytes: mechanisms and correlations to cell volume, hormone actions and metabolism. J Hepatol. 1996, **24 Suppl** 1:53-77.

11. Hilgemann DW, Yaradanakul A, Wang Y, Fuster D., Molecular control of cardiac sodium homeostasis in health and disease. *J Cardiovasc Electrophysiol*. 2006 May, **17 Suppl 1**: S47-S56.

12. Jain, S. K., Lim, G., Lipoic acid decreases lipid peroxidation and protein glycosylation and increases (Na(+)+K(+))- and Ca(++)-ATPase in high glucose-treated human reythrocytes. *Free Radic Biol Med.* 2000, **29**:1122–8.

13. Jain, S. K., Lim, G., Pyridoxine and pyridoxamine inhibits superoxide radicals and prevents lipid peroxidation, protein glycosylation, and (Na^++K^+)-ATPase activity reduction in high glucose-treated human erythrocytes. *Free Radic Biol Med.* 2001, **30**:232– 7.

14. Jeffcoate, S. L., Diabetes control and complications: the role of glycated haemoglobin, 25 years on. *Diabet Med*. 2004, **21**: 657–665.

15. Kaloyianni**,** M., Bourikas, D., Koliakos, G., The effect of insulin on Na^+-H^+ antiport activity of obese and normal subjects erythrocytes. *Cell Physiol Biochem*. 2001, **11(5)**: 253-8.

16. Katyare, S. S., Kumthekar, M. M., Altered kinetic attributes of Na(+)+K(+)-ATPase activity in kidney, brain and erythrocyte membranes in alloxan-diabetic rats. *Indian J. Exp. Biol*. 1992, **30(1)**: 26-32.

17. Konukoglu, D., Kemerli, G. D., Sabuncu, T., Hatemi, H., Relation of erythrocyte Na^+–K^+-ATPase activity and cholesterol and oxidative stress in patients with type 2 diabetes mellitus. *Clin Invest Med.* 2003, **26**: 279–84.

18. Lang, F., Mechanisms and significance of cell volume regulation. *J Am Coll Nutr.* 2007 Oct, **26 (5 Suppl)** : 613S-623S.

19. Li, J., Eastman, A., Apoptosis in an interleukin-2-dependent cytotoxic T lymphocyte cell line is associated with intracellular acidification: Role of the Na(+)/H(+)-antiport. *J Biol. Chem*. 1995 Feb 17, **270(7)**: 3203-11.

20. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J., Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**: 265–75.

21. Marchesi, V. T., Palade, G. E., The localization of Mg–Na–K activated adenosine triphosphatase on red cell ghost membranes. *J Cell Biol.* 1967, **35**: 385– 404.

22. Matteucci, E., Giampietro, O., Erythrocyte sodium hydrogen antiport activity is not a predicator of diabetic nephropathy. *Horm Metab Res*. 1996, **28**: 388–93.

23. Matteucci, E., Rizvi, S. I., Giampietro, O., Erythrocyte sodium/hydrogen exchange inhibition by (-) epicatechin. *Cell Biol Int.* 2001, **25(8)**:771-6.

24. Mazzanti., L., Rabini, R. A, Testa, I., Bertoli, E., Modifications induced by diabetes on the physicochemical and functional properties of erythrocyte plasma membrane. *Eur J Clin Invest.* 1989, **19**: 84– 9.

25. Orlowski, J., Grinstein, S., Diversity of the mammalian sodium/proton exchanger SLC9 gene family. *Pflugers Arch*. 2004 Feb, **447(5)**: 549-65.

26. Pandey, K. B., Mishra, N., Rizvi, S. I., Myricetin may provide protection against oxidative stress in type 2 diabetic erythrocytes. *Z Naturforsch C*. 2009 Sep-Oct, **64(9-10)**:626-30.

27. Peck, M., D., Interaction of lipids with immune function: Biochemical effects of dietary lipids on plasma membranes. *J Nutr Biochem.* 1994, **5**: 466– 78.

28. Ramachandran, A., Snehalata, C., Problems specific of developing countries and effect of westernization. Type 2 diabetes—prediction and prevention. *London7 Wiley*. 1999, 325–45.

29. Rebelato, E., Abdulkader, F., Curi, R., Carpinelli, A. R., Control of the Intracellular Redox State by Glucose Participates in the Insulin Secretion Mechanism. PLoS ONE 2011, **6(8)**: e24507. doi:10.1371/journal

30. Renugadevi, J., Prabu, S. M., Quercetin protectsagainstoxidativestress-relatedrenaldysfunctionby cadmium in rats. *Experimental and Toxicologic Pathology*. 2010, **62**: 471–481.

31. Rizvi, S. I., and Mishra, N., Anti-oxidant effect of Quercetin on Type 2 diabetic erythrocytes. *Journal of Food Biochemistry. 20*09, **33(3)***:* 404-415.

32. Rizvi, S. I., Zaid, M. A., Impairment of sodium pump and Na/H exchanger in erythrocytes from non-insulin dependent diabetes mellitus patients: effect of tea catechins. Clin Chim Acta. 2005 Apr, **354(1-2)**:59-67.

33. Rizvi, S. I., Zaid, M. A., Intracellular reduced glutathione content

in normal and type 2 diabetic erythrocytes: effect of Insulin and epicatechin. *J Physiol Pharmacol.* 2001, **52:**483–488.

34. Rizvi, S. I., Zaid, M. A., Modulation of erythrocyte membrane Na/K-ATPase activity by insulin in normal and type2 diabetic patients. Evaluation of insulin-like role of (_)epicatechin. *Med Sci Res*. 1998, **26:**245– 7.

35. Suhail, M., Rizvi, S. I., Red cell membrane (Na^++K^+)-ATPase in diabetes mellitus. *Biochem Biophys Res Commun*. 1987, **146**: 179-86.

36. Tekin N., Akyuz F., Temel, H. E., NO levels in diabetes mellitus: Effects of L-NAME and insulin on LCAT, Na^+/K^+ ATPase activity and lipid profile. *Diab Met Syndr*: *Clin Res Rev*. 2011, doi:10.1016/j.dsx.2010.12.004.

37. Varashree, B.S., Bhat, G.P., Correlation of Lipid Peroxidation with Glycated Haemoglobin Levels in Diabetes Mellitus. *Online J Health Allied Scs.* 2011; **10(2)**:11.

38. Young, I., Cho, Michael P. Mooney, and Daniel J. Cho., Hemorheological Disorders in Diabetes Mellitus. *J Diabetes Sci Technol* 2008, **2(6)**:1130-1138.

39. Singh, M. P., and Kumar, V., Biodegradation of vegetable and agrowastes by *Pleurotus sapidus*: A noble strategy to produce mushroom with enhanced yield and nutrition. *Cell. Mol. Biol.* 2012, **58** (1): 1-7.

40. Pandey, V. K., Singh, M.P., Srivastava, A. K., Vishwakarma S. K., and Takshak, S., Biodegradation of sugarcane bagasse by white rot fungus *Pleurotus citrinopileatus. Cell. Mol. Biol.* 2012, **58** (1): 8-14.

41. Ruhal, A., Rana, J. S., Kumar S., and Kumar, A., Immobilization of malate dehydrogenase on carbon nanotubes for development of malate biosensor. *Cell. Mol. Biol.* 2012, **58** (1): 15-20.

42. Vishwakarma, S. K., Singh, M. P., Srivastava A.K. and Pandey, V. K., Azo dye (direct blue) decolorization by immobilized extracellular enzymes of *Pleurotus* species. *Cell. Mol. Biol.* 2012, **58** (1): 21-25.

43. Dash, S. K., Sharma, M., Khare, S. and Kumar, A., *rmpM* gene as a genetic marker for human bacterial meningitis. *Cell. Mol. Biol.* 2012, **58** (1): 26-30.

44. Bertoletti, F., Crespan, E. and Maga, G., Tyrosine kinases as essential cellular cofactors and potential therapeutic targets for human immunodeficiency virus infection. *Cell. Mol. Biol.* 2012, **58** (1): 31-43.

45. Sandalli, C., Singh, K., and Modak, M. J., Characterization of catalytic carboxylate triad in non-replicative DNA polymerase III (pol E) of *Geobacillus kaustophilus* HTA. *Cell. Mol. Biol.* 2012, **58** (1): 44-49.

46. Kaushal, A., Kumar, D., Khare, S. and Kumar, A., *speB* gene as a specific genetic marker for early detection of rheumatic heart disease in human. *Cell. Mol. Biol.* 2012, **58** (1): 50-54.

47. Datta, J. and Lal, N., Application of molecular markers for genetic discrimination of *fusarium* wilt pathogen races affecting chickpea and pigeonpea in major regions of India. *Cell. Mol. Biol.* 2012, **58** (1): 55-65.

48. Siddiqi, N. J., Alhomida, A. S., Khan, A. H. and Onga, W.Y., Study on the distribution of different carnitine fractions in various tissues of bovine eye. *Cell. Mol. Biol.* 2012, **58** (1): 66-70.

49. Ong, Y. T., Kirby, K. A., Hachiya, A., Chiang, L. A., Marchand, B., Yoshimura, K., Murakami, T., Singh, K., Matsushita, S. and Sarafianos, S. G., Preparation of biologically active single-chain variable antibody fragments that target the HIV-1 GP120 v3 loop. *Cell. Mol. Biol.* 2012, **58** (1): 71-79.

50. Singh, J., Gautam, S. and Bhushan Pant, A., Effect of UV-B radiation on UV absorbing compounds and pigments of moss and lichen of Schirmacher Oasis region, East Antarctica. *Cell. Mol. Biol.* 2012, **58** (1): 80-84.

51. Singh, V. P., Srivastava, P. K., and Prasad, S. M., Impact of low and high UV-B radiation on the rates of growth and nitrogen metabolism in two cyanobacterial strains under copper toxicity. *Cell. Mol. Biol.* 2012, **58** (1): 85-95.

52. Datta, J. and Lal, N., Temporal and spatial changes in phenolic compounds in response *Fusarium* wilt in chickpea and pigeonpea. *Cell. Mol. Biol.* 2012, **58** (1): 96-102.

53. Sharma, R. K., JAISWAL, S. K., Siddiqi, N. J., and Sharma, B., Effect of carbofuran on some biochemical indices of human erythrocytes *in vitro. Cell. Mol. Biol.* 2012, **58** (1): 103-109.

54. Singh, A. K., Singh, S. and Singh, M. P., Bioethics A new frontier of biological Science. *Cell. Mol. Biol.* 2012, **58** (1): 110-114.

55. Adedeji, A. O., Singh, K. and Sarafianos, S. G., Structural and biochemical basis for the difference in the helicase activity of two different constructs of SARS-CoV helicase. *Cell. Mol. Biol.* 2012, **58** (1): 115-121.

56. Singh, S., Choudhuri, G., Kumar, R. and Agarwal, S., Association of 5, 10-methylenetetrahydrofolate reductase C677T polymorphism in susceptibility to tropical chronic pancreatitis in North Indian population. *Cell. Mol. Biol.* 2012, **58** (1): 122-127.

57. Sharma, R. K., Rai, K. D. and Sharma, B., *In* vitro carbofuran induced micronucleus formation in human blood lymphocytes. *Cell. Mol. Biol.* 2012, **58** (1): 128-133.

58. Naraian, R., Ram, S., Kaistha S. D. and Srivastava J., Occurrence of plasmid linked multiple drug resistance in bacterial isolates of tannery effluent. *Cell. Mol. Biol.* 2012, **58** (1): 134-141.

59. Pandey, A. K., Mishra, A. K., And Mishra, A., Antifungal and antioxidative potential of oil and extracts, respectively derived from leaves of Indian spice plant *Cinnamomum tamala. Cell. Mol. Biol.* 2012, **58** (1): 142-147.

60. Kumar, A., Sharma, B. and Pandey, R. S., Assessment of stress in effect to pyrethroid insecticides, λ-cyhalothrin and cypermethrin in a freshwater fish, *Channa punctatus* (Bloch). *Cell. Mol. Biol.* 2012, **58** (1): 153-159.

61. Srivastava N., Sharma, R. K., Singh, N. and Sharma, B., Acetylcholinesterase from human erythrocytes membrane: a screen for evaluating the activity of some traditional plant extracts. *Cell. Mol. Biol.* 2012, **58** (1): 160-169.

62. Singh, M.P., Pandey, A. K., Vishwakarma S. K., Srivastava, A. K. and Pandey, V. K., Extracellular Xylanase Production by *Pleurotus* species on Lignocellulosic Wastes under *in vivo* Condition using Novel Pretreatment. *Cell. Mol. Biol.* 2012, **58** (1): 170-173.

63. Kumar, S., Sharma, U. K., Sharma, A. K., Pandey, A. K., Protective efficacy of *Solanum xanthocarpum* root extracts against free radical damage: phytochemical analysis and antioxidant effect. *Cell. Mol. Biol.* 2012, **58** (1): 174-181.

64. Shukla, A., Singh, A., Singh, A., Pathak, L.P., Shrivastava, N., Tripathi, P. K., Singh, K. and Singh, M.P., Inhibition of *P. falciparum* pfATP6 by curcumin and its derivatives: A bioinformatic Study. *Cell. Mol. Biol.* 2012, **58** (1): 182-186.

65. Michailidis, E., Singh, K., Ryan, E. M., Hachiya, A., Ong, Y. T., Kirby, K. A., Marchand, B., Kodama, E. N., Mitsuya, H., Parniak, M.A. and Sarafianos, S.G., Effect of translocation defective reverse transcriptase inhibitors on the activity of n348i, a connection subdomain drug resistant HIV-1 reverse transcriptase mutant. *Cell. Mol. Biol.* 2012, **58** (1): 187-195.

66. Parveen, A., Rizvi, S. H. M., Gupta, A., Singh, R., Ahmad, I., Mahdi, F., and Mahdi, A. A., NMR-based metabonomics study of sub-acute hepatotoxicity induced by silica nanoparticles in rats after intranasal exposure. *Cell. Mol. Biol.* 2012, **58** (1): 196-203.