



# Asian Journal of **Biochemistry**

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## Nucleoside Degradation in Some *Streptomyces* Strains

Nadia H. Ali and Latifa A. Mohamed

Five strains of *Streptomyces* were screened for the abilities of their extracts to catalyze the hydrolytic or deaminating activities of purine and pyrimidine ribonucleosides and their bases. These studies are rare in *Streptomyces*. No hydrolytic cleavage for N-glycosidic bond of nucleosides was observed in all screened strains. Hydrolytic deamination was the only degradative activity occurred with cytidine (as substrate) from the ribonucleosides and their bases tested. *Streptomyces hygrosopicus* NRRL B-1476 gave the highest level of the hydrolytic deamination of cytidine to uridine. Uridine was chromatographically identified in cell-free extracts. Optimum pH and temperature of the enzyme activity were determined at 7.0 and 50°C, respectively. Thermal stability experiments indicated that the enzyme completely restored its activity at 50°C for 30 min, however a complete loss in enzyme activity was recorded when the enzyme was incubated at 80 and 90°C for 20 and 5 min, respectively. Dialyzed extract caused an increase in enzyme activity of about 55%. Results obtained indicate the involvement of sulfhydryl group(s) in the catalytic site of the enzyme. HgCl<sub>2</sub>, CuSO<sub>4</sub> and FeCl<sub>3</sub> (10<sup>-2</sup> M) caused a complete inhibition of enzyme activity, whereas, little enzyme activity was retained in presence of AgCl<sub>2</sub>, MgSO<sub>4</sub>, BaCl<sub>2</sub> and NaCl. Inhibition by uridine was of the competitive type and the enzyme exhibited classic Michaelis Menten saturation kinetics. Its apparent K<sub>m</sub> and K<sub>i</sub> values were found to be 4.16 and 21.9 mM, respectively. (*Asian Journal of Biochemistry* 3 (1): 1-10, 2008; doi: 10.3923/ajb.2009.1.10)

## Modulation of UVB-induced Oxidative Stress by Ursolic Acid in Human Blood Lymphocytes

S. Ramachandran, N. Rajendra Prasad, K.V. Pugalendi and Venugopal P. Menon

UV radiation-induced damages may result in pre-cancerous and cancerous lesions and acceleration of skin aging. It involves an imbalance of the endogenous antioxidant system that leads to the increase of free radical levels. Antioxidant pretreatment might inhibit such imbalance. In the present study, the photoprotective effect of ursolic acid (UA; 3β-hydroxy-urs-12-en-28-oic acid), a dietary polyphenolic phytochemical, has been examined in the UVB-(280-320 nm) irradiated human blood lymphocytes. Lymphocytes pretreated with increasing

concentrations of ursolic acid (1, 5 and 10  $\mu\text{g mL}^{-1}$ ) for 30 min, were irradiated and lipid peroxidation and antioxidant defense were examined. UVB-irradiated lymphocytes exhibited increased levels of lipid peroxidation and disturbances in antioxidant status. Ursolic acid pretreatment resulted in significant reduction in thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LPH) levels. Further, antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), vitamin-C (Vit-C) and vitamin-E (Vit-E) were normalised in ursolic acid pretreated plus UVB-treated lymphocytes. The maximum dose of ursolic acid (10  $\mu\text{g mL}^{-1}$ ) normalized the UVB induced lipid peroxidation, indicating the photoprotective effect of ursolic acid in human peripheral lymphocytes under *in vitro* condition. (*Asian Journal of Biochemistry* 3 (1): 11-18, 2008; **doi**: 10.3923/ajb.2009.11.18)

### **Adverse Hepatic Effects Associated with Administration of Antiretroviral Drugs (Nevirapine, Lamivudine and Stavudine) to Albino Rats: Implication for Management of Patients with HIV/AIDS**

R.A. Umar, S.W. Hassan, M.J. Ladan, I.K. Matazu, B. Shehu, R.A. Shehu, L.G. Muhammed and F.I. Molabo

We studied the effects of acute and sub-chronic oral administration of nevirapine, lamivudine and stavudine on liver function in albino rats. Acute administration of nevirapine resulted in significant ( $p < 0.05$ ) increases in activities of Aspartate amino transferase (AST) and Alanine amino transferase (ALT). Total proteins, albumin and globulin were significantly lowered. Upon sub-chronic administration of nevirapine, only AST and ALT activities were significantly raised. Acute administration of lamivudine was associated with significantly ( $p < 0.05$ ) lower albumin and globulin and higher total bilirubin and conjugated bilirubin levels. There were no significant differences ( $p > 0.05$ ) in liver function profiles associated with sub-chronic administration of the drug. However, acute and sub-chronic administrations of stavudine were not associated with significant ( $p > 0.05$ ) changes in liver function profiles. We conclude that while the use of stavudine is safe, acute and sub-chronic oral administration of nevirapine and lamivudine are associated with hepatotoxicity and hepatoprotective agents should be incorporated in the treatment regimens employing these drugs to avert life-threatening complications. (*Asian Journal of Biochemistry* 3 (1): 19-25, 2008; **doi**: 10.3923/ajb.2009.19.25)

## **Biochemical Composition and Fatty Acid Profile of the Green Alga *Ulva reticulata***

Annian Shanmugam and Chendur Palpandi

The total lipid, protein, carbohydrate, water and fatty acid contents of seaweed *U. reticulata* belonging to the class of Chlorophyceae that had been collected from Vellar estuary, southeast coast of India. Water contents of the *U. reticulata* was found as 75.33%. Carbohydrate, protein and lipid contents have been estimated as 50.248, 8.484, 19.98 and 1.7% of the dry sample correspondingly. The analysis of fatty acids by gas chromatography revealed the presence of myristic acid, palmitic acid, heptadecenoic acid, oleic acid and linoleic acid. Among the fatty acids, palmitic acid was predominant in all the fatty acids studied. (*Asian Journal of Biochemistry* 3 (1): 26-31, 2008; doi: 10.3923/ajb.2009.26.31)

## **Oxidative Stress in the Liver of Diabetic Rats Treated with a Combination of Sildenafil Citrate and a Free Radical Scavenger**

N.M. Abdel-Hamid, L.M. Faddah, M.A. Al-Rehany and A.A. Awad

Erectile Dysfunction (ED) is a common problem within diabetic patients. The liver is one of the most affected vital organs by diabetic consequences. Oxidative stress is the most known intermediary pathway initiating liver diseases among diabetics. The present study was designed to investigate the protective effect of alpha-tocopherol ( $\alpha$ -TP) against possible oxidative stress, that may be elicited by administration of Sildenafil Citrate (SC) and to assess whether SC may negatively affect the liver in an experimental diabetic model. SC was given to groups of normo-glycemic and diabetic rats, either alone or in combination with  $\alpha$ -TP, by oral route for two weeks. Hepatic tissue content of malondialdehyde-a thiobarbituric acid reactive oxygen species (TBARS) and reduced glutathione (GSH) were determined as biomarkers for oxidative stress in liver tissue. TBARS was significantly up-regulated in diabetic than normo-glycemic rats. SC significantly down-regulated TBARS content, an effect which was synergized by  $\alpha$ -TP co-administration. SC treatment depleted GSH in both normo-glycemic and hyperglycemic rats, this effect was completely reversed by  $\alpha$ -TP co-administration.  $\alpha$ -TP could not correct the effect of diabetes on liver GSH and TBARS contents and it couldn't restore these parameters in diabetic to non-diabetic values. In conclusion, our study explored the usefulness of  $\alpha$ -TP co-administration in protecting the liver against GSH depletion, induced by SC administration. We also elucidated that SC down-regulated TBARS in liver tissue,

an effect which was potentiated by  $\alpha$ -TP co-administration. We recommend the use of  $\alpha$ -TP as an adjuvant therapy to SC, specially for diabetic patients who are considered to be the most extensive users of the drug. (*Asian Journal of Biochemistry* 3 (1): 32-37, 2008; **doi**: 10.3923/ajb.2009.32.37)

### **A Modified Spectrophotometric Micromethod to Determine Serum Copper**

Mungli Prakash and Jeevan K. Shetty

Most of the available spectrophotometric methods to determine serum copper requires large volume of sample and involves several steps. We modified literature known method to estimate serum copper. Guanidine hydrochloride was used to release ceruloplasmin bound copper and determined using bathocuproine disulphonate disodium salt (BCDS). Our modified micromethod correlated well with the literature known method and shown good recovery and precision. Modified spectrophotometric micromethod is simple, sensitive, rapid, requires very less sample and coloring reagent and can be adopted in any clinical laboratory setups. (*Asian Journal of Biochemistry* 3 (1): 38-42, 2008; **doi**: 10.3923/ajb.2009.38.42)

### **The Hypocholesterolemic Effect of *Gariss* and *Gariss* Containing Bifidobacteria in Rats Fed on a Cholesterol-Enriched Diet**

Abdallah A. Elayan, Abdel Moneim E. Sulieman and F.A. Saleh

The effect of fermented camel milk *Gariss* and *Gariss* supplemented with *Bifidobacterium lactis* (Bb-12) on plasma and liver lipids was determined in rats fed on a cholesterol enriched diet. The two groups decreased the levels of plasma total cholesterol and the Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL) cholesterol than the positive control group. *Gariss* containing Bb-12 was more effective in the lowering of plasma and liver cholesterol levels than *Gariss* without bifidobacterium. (*Asian Journal of Biochemistry* 3 (1): 43-47, 2008; **doi**: 10.3923/ajb.2009.43.47)

### **Serum Total Thiol Status in Alcohol Abusers**

Mungli Prakash, Jeevan K. Shetty, Sudeshna Tripathy, Manish Verma, Saddinamane Vasudev and Panambur V. Bhandary

Thiol (-SH) groups are the major intracellular and extracellular reducing agents. This study estimates such thiol groups in alcohol abusers. Serum total thiols and liver function test parameters were estimated by spectrophotometric methods in alcohol abusers on admission (group I) and thirty days after alcohol abstinence along with life style modification (group II) and in non-alcoholic healthy controls. Serum amino transaminases, gamma glutamyl transpeptidase levels were increased and total thiols, total proteins and albumin levels were decreased in group I cases compared to group II cases and controls. Total thiols status improved significantly along with transaminases and transpeptidase with thirty days of alcohol abstinence and life style modification. In conclusion, total thiol status is decreased in alcohol abusers and abstinence of alcohol along with life style modification improves thiol antioxidant status and liver function. (*Asian Journal of Biochemistry* 3 (1): 48-51, 2008; doi: 10.3923/ajb.2009.48.51)

### **Extracellular Nucleases of *Rhizopus stolonifer***

E.S. Rangarajan, R.A. Deshpande and V. Shankar

*Rhizopus stolonifer* produces two extracellular nucleases viz., nuclease Rsn and RNase Rs in a ratio of approximately 1:60, when grown on YPG medium. The purified nuclease Rsn is a high Mr (67 kDa), metal requiring multifunctional endonuclease with a substrate specificity in the order of ssDNA>dsDNA>>RNA. It cleaves DNA non-specifically but with RNA, shows high preference for adenylic acid linkages. RNase Rs, on the other hand, is a 28.2 kDa atypical member of RNase T2 family of cyclizing RNases which produces 2', 3' cyclic nucleotides as the major end product of RNA hydrolysis. The purification, characteristics and potential application of these enzymes are reviewed. (*Asian Journal of Biochemistry* 3 (2): 52-61, 2008; doi: 10.3923/ajb.2009.52.61)

### **2D and 3D QSAR: Modeling of TIBO Derivatives as Reverse Transcriptase 1 Inhibitors**

A. Thakur, B.K. Tiwari, M. Thakur, N.D. Pandey, S.S. Narvi, S. Thakur and A. Bharadwaj

This report describes QSAR and SAR studies on the Inhibition of Reverse Transcriptase (RT) by 79 TIBO (Tetrahydroimidazobenzodizepin-2-one) derivatives using both classical and unconventional physicochemical properties and quantum molecular descriptors along with indicator parameters. The application of a multiple linear regression analysis indicated that a combination of classical

physicochemical descriptors and the indicator parameters yielded a statistically significant model for the activity,  $\log 1/C$  (50% of inhibition concentration of TIBO derivatives for RTs). The final selection of a potential TIBO compound for the inhibition of Reverse Transcriptase is made by quantum molecular modeling. We have found that, among the a number of Quantum and modeling parameters, the electron density on the 9th atom correlated best with the activity. (*Asian Journal of Biochemistry* 3 (2): 62-78, 2008; **doi**: 10.3923/ajb.2009.62.78)

## **Molecular Modelling Analysis of the Metabolism of Maraviroc**

Fazlul Huq

Maraviroc (MVC) is a selective CCR5 antagonist with potent activity and favourable pharmacological properties against human immunodeficiency virus type 1 (HIV-1). Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G\* level) calculations show that MVC and its metabolites have large LUMO-HOMO energy differences ranging from 5.3 to 5.8 eV, indicating that the compounds would be kinetically inert. The molecular surfaces of all the compounds are found to abound in neutral regions so that they may be subject to lyophilic attacks. The surfaces are also found to possess some electron-rich and electron-deficient regions so that they may be subject to electrophilic and nucleophilic attacks as well. Nucleophilic attacks may be due to glutathione and nucleobases in DNA as a result of which depletion of glutathione and oxidation of nucleobases in DNA may occur. The former would induce oxidative stress and hence cellular toxicity whereas the latter would cause DNA damage. However, because of kinetic inertness of the molecules, the rates of such adverse reactions are expected to be low. (*Asian Journal of Biochemistry* 3 (2): 79-89, 2008; **doi**: 10.3923/ajb.2009.79.89)

## **Restoration of Altered Carbohydrate and Lipid Metabolism by Hyponidd, a Herbomineral Formulation in Streptozotocin-Induced Diabetic Rats**

P. Subash-Babu, S. Ignacimuthu and P. Stanely Mainzen Prince

The purpose of this study was to evaluate the influence of Hyponidd, a herbomineral formulation and its effect on key enzymes of glucose metabolism and lipid metabolism in STZ-induced diabetic rats. Female Wistar rats with a body weight of 180-200 g were used in this study. The rats were divided into seven groups of six rats each after the induction of STZ-diabetes: normal rats; normal

rats given Hyponidd (200 mg kg<sup>-1</sup> b. wt.); diabetic control; diabetic rats given Hyponidd (50 mg kg<sup>-1</sup> b. wt.); diabetic rats given Hyponidd (100 mg kg<sup>-1</sup> b. wt.); diabetic rats given Hyponidd (200 mg kg<sup>-1</sup> b. wt.); diabetic rats given glibenclamide (600 µg kg<sup>-1</sup> b. wt.). After 45 days of treatment, fasting blood glucose, plasma insulin, serum and tissue carbohydrate metabolizing enzymes and lipid profiles were determined in normal and streptozotocin induced-diabetic rats. Oral administration of Hyponidd for 45 days resulted in significant (p<0.05) reduction in blood glucose, serum and tissue glucose-6-phosphatase, fructose-1, 6-bis phosphatase, total cholesterol, triglyceride and free fatty acids level. At the same time there was a significant increase in the levels of plasma insulin, hexokinase and HDL cholesterol in streptozotocin induced-diabetic rats. The effect of Hyponidd was compared with an oral hypoglycaemic agent, glibenclamide. Present study shows, that Hyponidd significantly restored the altered carbohydrate and lipid metabolism by exerting a beneficial action against secondary complications associated with diabetes mellitus. (*Asian Journal of Biochemistry* 3 (2): 90-98, 2008; doi: 10.3923/ajb.2009.90.98)

### **Protective Effect of Metformin on Cardiac and Hepatic Toxicity Induced by Adriamycin in Swiss Albino Mice**

A.M. Aleisa, S.S. Al-Rejaie, S.A. Bakheet, A.M. Al-Bekairi, O.A. Al-Shabanah, Abdulhakeem Al-Majed, Abdulaziz A. Al-Yahya and S. Qureshi

Diabetes Mellitus (DM) is a chronic disease that is characterized by deterioration of glycemic control. The disease is known to be caused by imbalance between Reactive Oxygen Species (ROS) and antioxidant defense systems. Hyperglycemia is commonly observed in a wide variety of diseases, including cancer. Although, therapy against glycemic control is used in all these diseases, the diabetic cancer patients are on additional therapy with anticancer drugs. The objective of present study was to investigate if metformin, a very popular antidiabetic agent can avert the cardiac and hepatic toxicity caused by Adriamycin (ADR), which is a commonly used cytotoxic drug. The experimental protocol included oral treatment of mice with different doses (62.5, 125 and 250 mg kg<sup>-1</sup> day<sup>-1</sup>) of metformin for 7 days. Some mice in each group were injected i.p. with ADR (15 mg kg<sup>-1</sup>), 24 h prior to sacrifice. In each case animals were killed, 24 h after the last treatment, blood sample was collected and plasma was separated for analysis of AST, ALT and CK-MB. Liver and heart from the same animals were excised for analysis of proteins, nucleic acids, MDA and NP-SH. The results obtained revealed that pretreatment with metformin (i) reduced the ADR-induced increase in the concentrations of AST, ALT and CK-MB (ii) protected against the



ADR-induced increase of MDA and decrease of DNA and NP-SH in both cardiac and hepatic tissues. These results demonstrate that the treatment with metformin might be useful to protect cardiac and hepatic toxicity. The exact mechanism of action is not known, however; the inhibition of ADR-induced increase of plasma enzymes and MDA and depletion of DNA and NP-SH by metformin may be attributed to its antioxidant potentials, which are well known for the reduction of glycotoxins and general improvement in cellular dysfunction. The use of Metformin by cancerous diabetic patients on cytotoxic therapy will be a boon to avert the cardiac and hepatic toxicity. (*Asian Journal of Biochemistry* 3 (2): 99-108, 2008; doi: 10.3923/ajb.2009.99.108)

## **Molecular Modelling Analysis of the Metabolism of Udenafil**

Fazlul Huq

In present study, molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G\* level) calculations have been carried out to obtain information on relative toxicity of UDF and its metabolites. The results of the analyses show that UDF and its metabolites have LUMO-HOMO energy differences of the order of 4.1 to 4.2 eV so that the compounds would all be moderately inert kinetically. The molecular surfaces of UDF and NDUDF are found to abound in neutral green and electron-deficient blue regions so that they can be subject to lyophilic and nucleophilic attacks. The latter attack can be due to glutathione and nucleobases in DNA so that the two compounds may induce cellular toxicity due to glutathione depletion and DNA damage due to oxidation of nucleobases in DNA. The other two metabolites UDFM1 and UDFM3 abound in electron-rich red and yellow regions so that they are more likely to be subject to electrophilic attacks and may very well act as antioxidants. (*Asian Journal of Biochemistry* 3 (2): 109-117, 2008; doi: 10.3923/ajb.2009.109.117)

## **Rehabilitating Activity of Mangiferin in Benzo(a) Pyrene Induced Lung Carcinogenesis**

Peramaiyan Rajendran, Ramachandran Venugopal, Ganapathy Ekambaram, Arumugam Aadithya and Dhanapal Sakthisekaran

Cancer chemoprevention involves prevention, delay, or reversal of the process of carcinogenesis through ingestion of dietary or pharmaceutical agents. A large number of potential chemopreventive agents are known, some of which have

proven effective in clinical trials. These agents may function by a variety of mechanisms, directed at all major stages of carcinogenesis. One mechanism of particular note involves the inhibition of biosynthesis; of polyamines such as spermine, spermidine and putrescine are promising drug for chemoprevention. In the present study we evaluate chemopreventive efficacy of mangiferin against Benzo(a)Pyrene (B(a)P) induced lung carcinogenesis. Male Swiss albino mice strains were selected for the present investigation. Lung carcinoma was induced with B(a) P (50 mg kg<sup>-1</sup> body weight, orally) and the treatment was started by the oral administration of mangiferin (100 mg kg<sup>-1</sup> body weight). The modulatory effect of the mangiferin was examined on lung and liver to evaluate the level of polyamines, protein carbonyl, nucleic acid content and lipid peroxidation. Mangiferin significantly decreased the levels of polyamines, protein carbonyl, nucleic acid content and lipid peroxidation that were found to be increased in lung cancer bearing animals. Mangiferin could effectively inhibit B(a)P-induced lung carcinogenesis in albino mice by offering protection from protein damage and also by suppressing cell proliferation. (*Asian Journal of Biochemistry* 3 (2): 118-125, 2008; *doi*: 10.3923/ajb.2009.118.125)

### **The Effect of Sildenafil Citrate (Viagra) Combination with Vitamin E on Some Blood Neurotransmitters and Minerals in Diabetic Rats**

N.M. Abdel-Hamid, L.M. Faddah, M.A. Al-Rehany and A.A. Awad

Erectile Dysfunction (ED) is a prevalent problem among most of diabetic patients. This defect is extensively treated by Sildenafil Citrate (SC), which is a potent phosphodiesterase -5 inhibitor (PDE-5 I). The mechanism of SC in treating ED in an experimental diabetic model, in conjunction with a potent antioxidant, vitamin E (vit E) is to be elucidated. Here, we report that, whether alone or in combination with E, SC induces penile erection, mostly, through organized modulation of blood neurotransmitters, namely, Serotonin (Ser), Dopamine (Dop), Nor-Epinephrine (NEP) and Nitric Oxide (NO), in addition to potassium (K), controlled calcium (Ca) influx modulation, affecting neurotransmitters' release into the circulation, without apparent impact on sodium level (Na). In the present study, Ser, Dop, NEP and NO blood levels were together, significantly up-regulated in normoglycemic and more significantly in alloxan diabetic rats by SC treatment, an effect which was non-significantly prohibited by E combination. K level was elevated by SC in normo-glycemic, but decreased in diabetic rats. This later action was reversed by E, however, Na level was only significantly elevated in diabetic group, given SC combined with E, but Ca levels were non-significantly changed. These

actions of SC elucidate-in part-the mechanism of its action and necessitate care to its impact on diseases related to autonomic neurotransmission. (*Asian Journal of Biochemistry* 3 (2): 126-133, 2008; **doi:** 10.3923/ajb.2009.126.133)

### **Phytochemical Screening and Antibacterial Activity of *Tamarindus Indica* Pulp Extract**

M.G. Abukakar, A.N. Ukwuani and R.A. Shehu

The phytochemical analysis and antibacterial activity of aqueous pulp extract of *Tamarindus indica* were studied. The aqueous pulp extract of this plant was obtained using hot water extraction method. The antibacterial activity of aqueous pulp extract of this plant was carried out against four bacteria; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* by disc diffusion method. Phytochemical constituents present in the extract were found to include saponins (2.2%), alkaloids (4.32%) and glycosides (1.59%). Aqueous pulp extract of *Tamarindus indica* showed antibacterial activity against all the tested bacteria in the order of sensitivity as *Staphylococcus aureus*>*Escherichia coli*>*Pseudomonas aeruginosa* with the exception *salmonella typhi*. The antibacterial activity of aqueous pulp extract on *Staphylococcus aureus* was sensitive at 80, 120, 140, 160 and 180 mg mL<sup>-1</sup> of extract with 0.2, 0.3, 0.6, 0.8 and 10.0 mm zones of inhibition while *Escherichia coli* revealed 0.2, 0.2, 0.4 and 0.6 mm zones of inhibition at 120, 140, 160 and 180 mg mL<sup>-1</sup> of extract, respectively. *Pseudomonas aeruginosa* was only sensitive at 140, 160 and 180 mg mL<sup>-1</sup> of the extract with 0.4, 0.6 and 0.8 mm zones of inhibition. (*Asian Journal of Biochemistry* 3 (2): 134-138, 2008; **doi:** 10.3923/ajb.2009.134.138)

### **Effect of Atorvastatin on Paraoxonase Activity in Patients with Hyperlipidemia**

Jeevan K. Shetty, Mungli Prakash, Sudeshna Tripathy, Manish Verma and Pannuri Vikram

Paraoxonase is high density lipoprotein associated enzyme which prevents the oxidation of low density lipoprotein. In the present study we evaluated the effect of atorvastatin on serum paraoxonase levels in south Indian population with hyperlipidemia. The study was conducted on 59 newly diagnosed hyperlipidemic patients and 41 healthy controls. Hyperlipidemic patients were divided into two groups, group 1-before treatment and group 2-three months after receiving 10 mg

atorvastatin daily. Serum paraoxonase and lipid profile were estimated in both cases and controls. Serum paraoxonase activity and high density lipoprotein levels were lower and total cholesterol, triglycerides and low density lipoproteins were high in hyperlipidemics compared to controls ( $p < 0.01$ ). Serum paraoxonase activity and high density lipoproteins levels were increased and total cholesterol, triglycerides and low density lipoprotein levels were decreased significantly in group 2 cases compared to group 1 cases ( $p < 0.01$ ). Serum paraoxonase correlated positively with high density lipoprotein ( $R = 0.347$ ,  $p < 0.01$ ). Atorvastatin apart from favorably improving lipid profile, also improves paraoxonase activity significantly, this may suggest anti-atherogenic role of statins along with their antilipidemic function. (*Asian Journal of Biochemistry* 3 (2): 139-142, 2008; doi: 10.3923/ajb.2009.139.142)

### **Age and Gender Related Changes in Free Radical Pathology and Antioxidant Defense in Schizophrenia**

P. Uma Devi and P. Chinnaswamy

The aim of the present study was to estimate the effect of age and gender on the levels of primary and secondary antioxidants and Malondialdehyde in red blood cells of the selected Schizophrenia patients. In our present study, the activities of six free radical scavenging enzymes (super oxide dismutase (SOD), catalase (CAT)), glutathione peroxidase (GSH-Px), glutathione Transferase (GST), glucose-6-phosphate dehydrogenase (G6PD), Caeruloplasmin ferroxidase (Cp) and the level of thiobarbituric acid-reactive substances (TBARS) as an index of lipid per oxidation were analyzed in the different age groups of schizophrenia patients. Role of gender was also analyzed in both schizophrenia and control subjects. It was observed from the results that there was a significant increase in erythrocyte MDA levels and activity of SOD and a significant decrease in erythrocyte CAT, GSH-Px, Cp-ferroxidase and G6PD levels in patients with schizophrenia, when compared to controls ( $p < 0.01$ ). The results have also shown that among different age groups, highly significant oxygen free radical production, evidenced by increased levels of MDA and decreased levels of antioxidant enzymes activity was found in adult and elderly schizophrenia patients, which supports the more pronounced oxidative stress in adult and elderly schizophrenia patients when compared to young schizophrenia patients. The statistically more significant increase ( $p < 0.001$ ) in the activity of SOD in elderly schizophrenia subjects may be a compensatory regulation in response to increased oxidative stress in elders. The decreased concentrations of the CAT, GSH-Px, G6PD and Cp-ferroxidase support the hypothesis that lipid per oxidation is an important

causative factor in the pathogenesis of schizophrenia. These data reveal that antioxidant defense mechanisms might be impaired a lot in normal elderly people and schizophrenia patients with age group above 30 (i.e., adults and elders). As for as gender concerned, we observed a significant raise in the levels MDA, SOD and significant decrease in the levels of selected antioxidant enzymes in schizophrenia male and female subjects when compared with the respective control male and female subjects ( $p < 0.01$ ). But we found statistically more significant increase in the levels of MDA and highly significant decrease in the levels of the secondary antioxidant enzymes G6PD and Cp ferroxidase ( $p < 0.001$ ) were found in schizophrenia males when compared with schizophrenia females. Supplementation of antioxidants may prevent further oxidative injury in elderly schizophrenia patients. (*Asian Journal of Biochemistry* 3 (3): 143-152, 2008; doi: 10.3923/ajb.2009.143.152)

### ***In vitro* Genotoxic Effect of Anaesthetic Halothane on Rabbit Lymphocytes and the Protective Role of Vitamin A Supplementation**

Hussein I. El-Belbasi, Hany M. Hassan and Mona M. Mantawy

The target of the present study is to estimate the *in vitro* genotoxic potential of the volatile-anaesthetic halothane (Narcotan<sup>®</sup>) as well as the protective effect of vitamin A, as an antioxidant. To achieve this task, the alkaline single cell gel electrophoresis (comet assay) was applied as the extent of DNA fragmentation in rabbit lymphocytes that were exposed *in vitro* to halothane at concentrations of 0.1 or 1.0 mM for 10 and 30 min, respectively. The obtained results elucidated that anaesthetic halothane induced a significant increase in DNA damage that shown by comet DNA concentration and its tail length. The recorded elevation of DNA damage was clearly correlated with halothane concentration as well as the period of exposure. To mend the deleterious effect of halothane on DNA, 25 rabbits were orally received vitamin A (8000 IU kg<sup>-1</sup> body weight) for 15 days prior to *in vitro* halothane exposure. Vitamin A administration induced a significant repairing effect in lymphocytes-damaged DNA due to halothane exposure, comparing with the non-administrated group. The genotoxic effect of halothane might be attributed to its property as an oxidative agent capable to accumulate oxygen free radicals inside the cell and producing DNA damage. Thereby, the antioxidant property of vitamin A might counteract the hazardous effect of anaesthetic halothane. It is concluded that there is a great need of vitamin A intake for patients undergoing surgical operations with halothane anaesthesia and as a prophylactic therapy for the operation room personnel to antagonize the possible

genotoxic effect of halothane. (*Asian Journal of Biochemistry* 3 (3): 153-161, 2008; **doi:** 10.3923/ajb.2009.153.161)

### **Toxicity Biosensor for the Evaluation of Cadmium Toxicity Based on Photosynthetic Behavior of Cyanobacteria *Anabaena torulosa***

Wong Ling Shing, Salmijah Surif and Lee Yook Heng

A biosensor based on electrochemical transduction using an oxygen probe has been developed for the measurement of Cd<sup>2+</sup> toxicity by using cyanobacteria (*Anabaena torulosa*), where the cells were immobilized on the surface of an oxygen probe. The biosensor responded to the changes in photosynthetic oxygen release under illumination by a light source. Exposure to Cd<sup>2+</sup> at concentrations below approximately 8 mg L<sup>-1</sup> did not demonstrate any inhibition but stimulation of oxygen production was observed. Inhibition occurred only when concentration of Cd<sup>2+</sup> was above 8 mg L<sup>-1</sup>. Using the same concentration range of Cd<sup>2+</sup> but increasing the exposure time of Cd<sup>2+</sup> from 15 to 30 min, inhibition of oxygen release was mainly observed. This pattern of inhibition of *A. torulosa* by Cd<sup>2+</sup> was different from that of Cu<sup>2+</sup>, which was also measured by the same toxicity biosensor, where no stimulation in oxygen release was observed. The use of toxicity biosensor has enabled the detail toxicity behaviour of *A. torulosa* towards Cd<sup>2+</sup> to be evaluated. (*Asian Journal of Biochemistry* 3 (3): 162-168, 2008; **doi:** 10.3923/ajb.2009.162.168)

### **Immobilization of $\alpha$ -Amylase from Acha (*Digiteria exilis*) on Different Cellulose Fibre Materials**

E.C. Egwim and O.B. Oloyede

Alpha amylase was obtained from Acha (*D. exilis*) sprouted for 96 h and immobilized on palm wood chips, coconut and cotton wood fibers according to standard procedures. Palm wood chips maintained enzyme activity up to 25 h and still retained about  $7 \times 10^{-2}$  mg glucose mL min<sup>-1</sup> of residual activity while coconut and cotton wool lost all activity by 12 h. Enzyme half-life improved by 857.0, 21.4 and 28.6 folds using palm wood chips, coconut and cotton wool fibre as carriers, respectively. Palm wood clip improved V<sub>max</sub> and catalytic efficiency by 177 and 163%, respectively. The results concludes that palm wood chip can be used to immobilize  $\alpha$ -amylase from *D. exilis* for several cycles of reuse. (*Asian Journal of Biochemistry* 3 (3): 169-175, 2008; **doi:** 10.3923/ajb.2009.169.175)

## ***In vitro* Antifungal Effect of EDTA Disodium Salt in Tested Black Aspergilli**

Luís Abrunhosa and Armando Venâncio

The antifungal effect of Na<sub>2</sub>EDTA on an *Aspergillus carbonarius*, an *A. ibericus*, an ochratoxigenic *A. niger* and a non-ochratoxigenic *A. niger* strain was studied. Also, the effect of Na<sub>2</sub>EDTA on the production of ochratoxin A by *A. carbonarius* and the ochratoxigenic *A. niger* was evaluated. The poisoned food technique was used with CYA medium supplemented with 0, 1 and 10 mmol L<sup>-1</sup> of Na<sub>2</sub>EDTA. The colony diameters were recorded daily and the amount of ochratoxin A produced was quantified every two days. Significant reductions of growth rates were observed in the presence of Na<sub>2</sub>EDTA being the calculated EC<sub>50</sub> of 2.1 mmol L<sup>-1</sup> for *A. carbonarius*, 0.9 mmol L<sup>-1</sup> for *A. ibericus*, 2.0 mmol L<sup>-1</sup> for the ochratoxigenic *A. niger* and 4.1 mmol L<sup>-1</sup> for the non-ochratoxigenic *A. niger*. Furthermore, 10 mmol L<sup>-1</sup> Na<sub>2</sub>EDTA delayed the production of ochratoxin A and reduced the levels in approximately 99% during 8 days. Na<sub>2</sub>EDTA is frequently used in the food industry and in agriculture agrochemicals and its effects on ochratoxigenic black aspergilli is not well known. This study showed that Na<sub>2</sub>EDTA can significantly reduce the growth rates of tested fungi and its ochratoxin A production. (*Asian Journal of Biochemistry* 3 (3): 176-181, 2008; **doi**: 10.3923/ajb.2009.176.181)

## **Complementary Hypoglycemic and Anti-Hyperglycemic Activity of Various Extracts of Fenugreek Seeds in Rats**

Mukesh Yadav, Radha Tomar, GBKS Prasad, Shalini Jain and Hariom Yadav

In present study, five extracts of fenugreek seed with different solvents (water, ethanol, methanol, hexane and chloroform) alone and in combination with glimepiride were tested for hypoglycemic and anti-hyperglycemic activity in rats by screening blood glucose for 6 h. Water extract exhibited highest hypoglycemic and anti-hyperglycemic activity in rats among all the extracts, while hexane and other extracts exhibited least and moderate activity, respectively. Water extract was further studied to dose dependent [200, 100 and 50 mg kg<sup>-1</sup> body weight (b.wt.)] hypoglycemic and anti-hyperglycemic effects alone and in combination with glimepiride (20, 10 and 5 mg kg<sup>-1</sup> b.wt.). The combination of water extract (200 mg kg<sup>-1</sup> b.wt.) and lower dose of glimepiride (5 mg kg<sup>-1</sup> b.wt.) has shown safer and potent hypoglycemic as well as anti-hyperglycemic activity and not created severe hypoglycemia in normal rats, while higher doses (200 mg kg<sup>-1</sup>

b.wt. of water extract and 10 and 20 mg kg<sup>-1</sup> b.wt. of glimepiride) were generated lethal hypoglycemia in normal rats. The results of present study enforced to say that, the water extract of fenugreek seeds has higher hypoglycemic and anti-hyperglycemic potential and may use as a complementary medicine to treat the diabetic hyperglycemia by significantly reducing dose of standard drugs. (*Asian Journal of Biochemistry* 3 (3): 182-187, 2008; doi: 10.3923/ajb.2009.182.187)

### **Ethanol Production from *Carica papaya* (Pawpaw) Fruit Waste**

B.C. Akin-Osanaiye, H.C. Nzelibe and A.S. Agbaji

The production of ethanol from *Carica papaya* (pawpaw) agricultural waste, using dried active baker's yeast strain (*Sacchromyces cerevisiae*) was investigated. The pawpaw fruit considered to be an agricultural waste was the tapped ripe pawpaw fruit harvested after the tapping of papain. The proximate composition, pH and the reducing sugar of the pawpaw fruit were determined. Effects of yeast concentration, duration of fermentation, pH, temperature and different yeast supplements as they relate to the optimization of the ethanol production were investigated. The fermented pawpaw fruit waste produced ethanol contents 2.82-6.60% (v/v). Proximate analyses of the dry fruit showed that pawpaw waste contain 90.82 g/100 g carbohydrate, 2.60 g/100 g lipid, 1.63 g/100 g crude protein, 4.95 g/100 g ash. The results of this work show that the rate of alcohol production through fermentation of pawpaw fruit waste by baker's yeast (*Sacchromyces cerevisiae*) increases with fermentation time and peaked at 72 h. It is also increased with yeast concentration at the temperature of 30°C. The optimum pH for fermentation is 4.5. (*Asian Journal of Biochemistry* 3 (3): 188-193, 2008; doi: 10.3923/ajb.2009.188.193)

### **Production of Extracellular $\alpha$ -amylase by *Streptomyces albidoflavus***

K.J.P. Narayana and M. Vijayalakshmi

A streptomycete strain, *Streptomyces albidoflavus* was isolated from soil and culture conditions were optimized for maximum production of  $\alpha$ -amylase under submerged fermentation. The optimum period for maximum amylase production was found to be 84 h. The suitable pH and temperature for amylase activity were 6.5 at 30°C. The levels of  $\alpha$ -amylase activity detected in culture filtrate varied greatly with type of carbon source used. Soluble starch stimulated  $\alpha$ -amylase yield



followed by trehalose and maltose. Nitrogen sources like yeast extract, tryptone, NaNO<sub>3</sub>, peptone and soybean meal were found to support the amylase production by the strain. The strain produced maximum amylase when medium contained starch and yeast extract at concentration of 1.5% (wt./vol.) and 0.2% (wt./vol.) respectively. (*Asian Journal of Biochemistry* 3 (3): 194-197, 2008; *doi*: 10.3923/ajb.2009.194.197)

### **Production of Extracellular Protease by *Streptomyces albidoflavus***

K.J.P. Narayana and M. Vijayalakshmi

Production of protease by *Streptomyces albidoflavus* isolated from laterite soil was studied under submerged fermentation. The strain started protease production after 24 h of incubation and maximum level of enzyme production was found with 72 h old culture. Attempts were made to optimize the cultural conditions for getting high yields of enzyme. The optimum levels of pH and temperature for enzyme production were 7.0 and 35°C respectively. Among carbon sources, maltose (1%) supported maximum production of protease followed by trehalose, glycerol, starch and glucose. High yield of protease was recorded in the medium supplemented with peptone (0.75%) followed by beef extract, casein, yeast extract, tryptone and NaNO<sub>3</sub>. (*Asian Journal of Biochemistry* 3 (3): 198-202, 2008; *doi*: 10.3923/ajb.2009.198.202)

### **Comparative Study on Characteristics of Seed Oils and Nutritional Composition of Seeds from Different Varieties of Tobacco (*Nicotiana tabacum* L.) Cultivated in Bangladesh**

M. Abbas Ali, M. Abu Sayeed, Rajesh Kumar Roy, Sarmina Yeasmin and Astaq Mohal Khan

The objectives of this study are to determine characteristics of seed oils and nutritional compositions of seeds from three varieties (Gidri, Virginia and Jati.) of tobacco. Present results revealed specific gravity (0.9235-0.9296), refractive index (1.4763-1.4815), pour point [-17-(-18)°C], flash point (135-142°C), fire point (154-162°C), cloud point [-15-(-18)°C], smoke point (215-230°C), cetane index (38.2-42.8), iodine value (134.37-138.20), saponification value (185.05-189.11), saponification equivalent (296.65-303.17), acid value (3.02-4.23), free fatty acid (1.51-2.12%), ester value (182.03-184.88), unsaponifiable matter (1.39-1.45%), peroxide value (1.77-2.56) and

Reichert-Meissl value (0.38-0.45). No significant differences ( $p < 0.05$ ) were observed among the varieties for refractive index, pour point, ester value, unsaponifiable matter and Reichert-Meissl value. High values ( $p < 0.05$ ) for thermal properties flash point, fire point and smoke point were found in Gidri seed oil while Jati seed oil had highest cetane index. Iodine and peroxide values were low in Gidri seed oil compared to other varieties. Jati had the lowest in saponification and acid values. Glyceride classes were estimated to be monoglycerides (1.01-1.15%), diglycerides (3.75-6.39%) and triglycerides (88.65-91.39%) whereas lipid classes to be neutral lipid (83.10-85.02%), glycolipid (7.16-11.36%) and phospholipid (5.15-6.98%). No significant differences ( $p < 0.05$ ) were observed among the varieties for triglyceride and neutral lipid contents. Saturated and unsaturated fatty acids present in the oils were separated and amounted to be (11.01-14.32%) and (82.73-86.75%), respectively, depending upon the varieties. GLC analysis showed the presence of significantly different ( $p < 0.05$ ) percentage of fatty acids from series palmitic ( $C_{16:0}$ ) to arachidic ( $C_{20:0}$ ). Linoleic acid was the principal ranging from 67.40-72.10%. Among the varieties Jati seed oil was highest in unsaturated fatty acids. All varieties contained large amounts of lipid (42.29-45.72%), protein (19.21-21.05%), crude fiber (14.58-16.89%) and other essential nutrients. Except for the ash content, there were significant differences ( $p < 0.05$ ) in the levels of all parameters among the sample tested. The knowledge of the present studies on different varieties of tobacco seeds could be important to its appropriate industrial use and for improvement in the nutritional value. (*Asian Journal of Biochemistry* 3 (4): 203-212, 2008; doi: 10.3923/ajb.2009.203.212)

### **Prevention of Carbon Tetrachloride ( $CCl_4$ )-Induced Liver Damage in Rats by *Acanthus montanus***

K.C. Patrick-Iwuanyanwu and M.O. Wegwu

The hepatoprotective effects of aqueous and alcoholic extracts of stem and leaf of *Acanthus montanus* pre-treatment against carbon tetrachloride ( $CCl_4$ )-induced liver damage in wistar albino rats were investigated. The plant extracts were fed to the rats intragastrically for 15 days prior to the administration of 0.5 mL  $CCl_4$   $kg^{-1}$  body weight. Serum L-alanine aminotransferase (L-ALT), L-aspartate amino transferase (L-AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels, 24 h after  $CCl_4$  administration decreased significantly in rats pre-treated with aqueous and alcoholic extracts of both stem and leaf of *Acanthus montanus* than in  $CCl_4$ -treated rats only. The aqueous extract of stem of *Acanthus montanus* showed a marked decrease in the levels

of AST, ALT and ALP when compared with the alcoholic extract of stem including aqueous and alcoholic extract of leaf of *Acanthus montanus*. However, rats fed with the alcoholic extract of leaf of *Acanthus montanus* was lowest in the level of LDH when compared with the results obtained from the other extracts of the stem and leaf of *Acanthus montanus*. Determination of total serum bilirubin also showed a remarkable decrease in rats pre-treated with aqueous and alcoholic extracts of stem and leaf of *Acanthus montanus* when compared with those administered CCl<sub>4</sub> alone. Aqueous leaf extract of *Acanthus montanus* showed the least result in total serum bilirubin when compared with the alcoholic extracts of the leaf and both aqueous and alcoholic extracts of the stem of *Acanthus montanus*. Lipid peroxidation expressed by malondialdehyde (MDA) concentration was significantly reduced in rats pre-treated with aqueous and alcoholic extracts of stem and leaf of *Acanthus montanus* than rats administered CCl<sub>4</sub>-alone. The lowest MDA concentration was observed in aqueous extracts of stem of *Acanthus montanus* while the highest concentration in rats pre-treated with aqueous and alcoholic extracts of stem and leaf of *Acanthus montanus* was observed in rats treated with alcoholic extract of stem of *Acanthus montanus*. Histopathological examinations in rats administered CCl<sub>4</sub>-alone showed severe hepatic damage to the liver. However, rats pre-treated with extracts of *Acanthus montanus* showed significant improvements in the architecture of rat liver. The results obtained in this study suggest that alcoholic and aqueous extracts of leaf and stem of *Acanthus montanus* may prevent liver damage induced by CCl<sub>4</sub> in rats. (*Asian Journal of Biochemistry* 3 (4): 213-220, 2008; doi: 10.3923/ajb.2009.213.220)

### **Antiobesity Effects of Pulp Extract *Tamarindus indica* in Albino Rat**

A.N. Ukwuani, M.G. Abukakar, R.A. Shehu and L.G. Hassan

The pulp extract of *Tamarindus indica* is used by traditional herbalists as a purgative, drug vehicle and antiobesity agent. The effect of 28 days administration of *Tamarindus indica* pulp extract on the body weight and lipid profile of the rat was evaluated. There was a significant increase in the weight of the control compared to the treated which significantly decreased ( $p < 0.05$ ) especially the rats given higher doses (2700 to 4500 mg kg<sup>-1</sup> body weight). Serum cholesterol and Low Density Lipoprotein (LDL) revealed a significant decrease ( $p < 0.05$ ) while High Density Lipoprotein (HDL) and triglycerides increased in the controlled group compared to the control. Xenical treated group was not significantly different ( $p < 0.05$ ) from the control. Triglycerides significantly increased ( $p < 0.05$ )

and LDL significantly decreased ( $p < 0.05$ ) in the pulp extract treated group as compared to xenical treated group. (*Asian Journal of Biochemistry* 3 (4): 221-227, 2008; doi: 10.3923/ajb.2009.221.227)

### **Biochemical Indices of Macrovascular Complication in Diabetic Rat Model: Compared Effects of *Vernonia amygdalina*, *Catharantus roseus* and Chlorpropamide**

M.U. Eteng, B.J. Bassey, I.J. Atangwho, G.E. Egbung, E.U. Eyong, P.E. Ebong and A.O. Abolaji

Hypoglycemic and biochemical effects of herbal extracts of *Vernonia amygdalina* Del. (Compositae), *Catharantus roseus* (L.) G. Don (Apocynaceae) and chlorpropamide were compared and status of macrovascular complications evaluated using biochemical indices in normal and diabetic rats. The phytochemistry of the herbs was also assessed. Hypoglycemic activity of the herbs and chlorpropamide was evaluated on diabetic and non-diabetic rats but biochemical effects of the treatments was evaluated only on diabetic rats, assigned into four study groups ( $n = 8$ ). Group I (control) received placebo (30% ethanol), treatment group II was gavaged with chlorpropamide in dose  $14.28 \text{ mg kg}^{-1}$  body weight, while groups III and IV were administered extracts of *V. amygdalina* ( $400 \text{ mg kg}^{-1}$  body weight) and *C. roseus* ( $400 \text{ mg kg}^{-1}$  body weight), respectively in 30% ethanol vehicle for 21 days. Results of the phytochemistry assessment identified alkaloids, cardiac glycosides, saponins, flavonoids, tannins in *V. amygdalina* and *C. roseus*. Triterpenes were identified only in *V. amygdalina* and anthroquinones only in *C. roseus*. All three treatments produced hypoglycaemic activity in normal and diabetic rats and significantly ( $p < 0.05$  to  $< 0.01$ ) reduced triglyceride and total Cholesterol relative to controls. *C. roseus* alone significantly ( $p < 0.01$ ) elevated HDL-Cholesterol. Serum protein significantly ( $p < 0.05$ ) increased in all treatments compared with controls. Urea levels decreased in all the treatments but more dramatic with chlorpropamide. Aminotransferase activity was not altered except serum ALT which was reduced in treated rats. Electrolyte profile showed dilutional hyponatremia with chlorpropamide treatment, which was absent in *C. roseus* treatment but mild in *V. amygdalina*. These changes in biochemical indices of toxicity and macrovascular complications are discussed with respect to the comparative therapeutic benefits of the three treatments. (*Asian Journal of Biochemistry* 3 (4): 228-234, 2008; doi: 10.3923/ajb.2009.228.234)

## **Confirmation of non N-glycan Linked Mannose Glycosylation in Chitinase 42 kDa Secreted by *Trichoderma harzianum* BIO10671**

M. Muskhazli, Q.Z. Faridah, R. Salfarina, T. Nor Farizan, I. Nalisha and G.L.F. Wallis

Chitinase 42 kDa produced by *Trichoderma harzianum* has been proven as a prime compound to be excreted onto the hyphae of the pathogen causing localised cell wall lysis at the point of interaction. This finally initiate the process of the host cell becomes empty of cytoplasm, disintegrates and shows a rapid collapse. This study investigates the existence of N-glycan linked mannose in chitinase 42 kDa produced by the Malaysian *T. harzianum* strain BIO10671. The chitinase 42 kDa from *T. harzianum* BIO10671 was initially purified using anion exchange chromatography prior to a series of experiments such as immunoblotting against the chitinase 42 kDa antibody, lectin staining for detecting any terminal linked mannose and galactofuranose detection to determine the presence of galactofuranose components in glycoproteins. The enzyme purification harvested about 12-fold of chitinase 42 kDa from *T. harzianum* BIO10671 with strong indication of the presence chitinase 42 kDa presence on SDS-Page. This was confirmed by immunoblotting with a strong response around 42 kDa after overnight incubation in chitinase 42 kDa antibody suggesting that the gene for chitinase 42 kDa was greatly expressed in this strain. There are no intervention of galactofuranose on any of the terminal mannose in chitinase 42 kDa as shown by negative results on samples treated with or without endoglycosidase-H and lectin staining. Therefore, it can be concluded that glycosylation occurred in the chitinase 42 kDa from *T. harzianum* 42 kDa was not in the form of N-glycan linked mannose as expected. (*Asian Journal of Biochemistry* 3 (4): 235-242, 2008; *doi*: 10.3923/ajb.2009.235.242)

## **Isolation, Purification and Characterization of Lipase from Grey Mullet (*Liza parsia* Hamilton, 1822)**

M.A. Islam, N. Absar and Abdus Salam Bhuiyan

Two lipases designated as Lip-1 and Lip-2 were purified from dorsal part of grey mullet (*Liza parsia*) to homogeneity by 85% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fractionation followed

by simultaneous desalting and concentration by ultrafiltration and Sephadex G-50 and DEAE-cellulose chromatography and CM-cellulose chromatography. The molecular weight of two lipases was determined by SDS-PAGE and gel filtration about 46.5 and 41.2 KDa, respectively. Both the enzymes were dimer in nature remained unchanged the presence and absence of reducing agent under SDA-PAGE. The Lip-1 and Lip-2 lipases were active within the pH range of 7-8.5, with an optimum pH of 8 and 8.5.0 and were stable from 2.0-10.5. The enzyme was active within the temperature range of 30-60°C and maximum activities were observed around 33 and 35°C, respectively and beyond which it lost activity progressively. The hydrolytic activity was enhanced by Ca<sup>+</sup> and EDTA (concentration 0.001-0.003 M) but strongly inhibited by heavy metal s Cd<sup>++</sup>, Zn<sup>++</sup> and Hg<sup>++</sup>. The presence of Zn<sup>++</sup> and Hg<sup>++</sup> potently inhibited lipolytic activities of the lipases from grey mullet, while activities were slightly inhibited in the presence of Cu<sup>++</sup> salts. (*Asian Journal of Biochemistry* 3 (4): 243-255, 2008; *doi*: 10.3923/ajb.2009.243.255)

## **Physicochemical Characterisation of Heat and Cold Pressed Peanut Meal Flours**

Kain Regena Juliana and Chen Zhengxing

The functional and chemical properties of peanut meal flours obtained as by-products from both cold and heat pressed peanut oil extraction methods were studied. Peanut meal flour was found to consist mostly of protein (~51%) with lower starch content (~24%). The processing methods were found to have influenced functional properties such as water and oil absorption, emulsification, foam formation and stability. The neutral sugar components were mostly glucose, galactose and mannose with minute quantities of xylose, arabinose and rhamnose. High pressing temperatures, during the extraction process, resulted to the decomposition of some polysaccharides into other neutral sugar components; hence the significantly high percentage of xylose and arabinose in the polysaccharides extracted from the heat pressed peanut flour. Extraction methods also had profound influence on the fatty acid and amino acid composition of peanut flours obtained. However, flours obtained from the different methods exhibited adequate functional properties that could be usefully employed in different food formulations. (*Asian Journal of Biochemistry* 3 (4): 256-266, 2008; *doi*: 10.3923/ajb.2009.256.266)

## **The Hypocholesterolemic Effect of *Stachytarpheta cayennensis* Tea: Implications for the Management of Obesity and Hypertension**

Ikewuchi Jude Chigozie, Osaghae Blessing Eloghosa and Osagie Augusta

The effects of *Stachytarpheta cayennensis* tea on the weight and plasma total cholesterol concentration of normal rabbits were investigated. Daily oral administration ( $2 \text{ mL kg}^{-1}$ ) of the tea led to significant decreases ( $p < 0.05$ ) in the weight and plasma total cholesterol concentrations of the rabbits. These results support the use of *S. cayennensis* tea in the management of hypertension and obesity. (*Asian Journal of Biochemistry* 3 (4): 267-270, 2008; doi: 10.3923/ajb.2009.267.270)

## **Effect of Feeding High-Fat with or Without Sucrose on the Development of Diabetes in Wistar Rats**

S. Sivabalan and Venugopal P. Menon

The aim of the study is to know the effect of high-fat and sucrose in the development of type 2 diabetes in Wistar rats. Several factors including high caloric diets, chronic positive caloric balance and the sedentary lifestyles are proposed as the causative factors in the development of type 2 diabetes but the exact mechanism involved is not known. Numerous studies on genetic models of obesity and diabetes induced by chemical destruction of pancreas are available, but the metabolic syndrome and diabetes observed in general population is mostly different from the genetic models and chemically induced diabetes. In this study we analyzed the effect of feeding, high-fat with or without sucrose, orally for 12 weeks. The rats were divided into three groups of twelve animals each. The groups are control, high-fat and high-fat with sucrose. After 12 weeks of treatment, fasting glucose, fasting plasma insulin, glucose tolerance test, plasma triglycerides, cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol were analyzed. Insulin resistance was analyzed by euglycemic clamp technique. Both high-fat diet and high-fat diet with sucrose impaired the lipid profile, glucose tolerance and have caused severe insulin resistance in the normal Wistar rats. (*Asian Journal of Biochemistry* 3 (5): 271-279, 2008; doi: 10.3923/ajb.2009.271.279)

## **Buffering Capacity of Saliva in Patients with Active Dental Caries**

Mohammad Reza Malekipour, Manoochehr Messripour and Farzaneh Shirani

Saliva buffer act as an important factor to control the pH of the mouth environment. Because organic acids produced by the mouth microorganisms is associated with development of dental caries, the aim of this study was to compare the buffering capacity of saliva in active dental caries patients with caries free subjects. Saliva samples were collected without stimulation from 30 patients with more than 10 decayed teeth and 30 subjects with no dental caries. The pH of saliva was measured and the buffering capacity of each saliva sample was determined by either HCl (1:10 N) or NaOH (1:10 N) titration. The determination of pH values of patients with active caries and caries free subjects were  $6.67\pm 0.03$  and  $6.76\pm 0.03$ , respectively which are not significantly different. However, the pattern of titration of the saliva in the patients was different from that of titration of the healthy subject. The differences were significant particularly after addition of 1-3 mL of either HCl or NaOH solution. The result suggested that the determination of the buffering capacity of the saliva may be used as an index for the development of dental caries. (*Asian Journal of Biochemistry* 3 (5): 280-283, 2008; *doi*: 10.3923/ajb.2009.280.283)

## **Starch Hydrolysis and $\alpha$ -Amylase Activity of *Aspergillus* and *Chaetomium***

Dhruba Sharma and A.K. Shukla

Starch hydrolyzing and  $\alpha$ -amylase producing micro-fungi were isolated from soil. Among the isolated fungal species *Aspergillus fumigatus* and *Chaetomium globosum* were found prominent starch hydrolyzing and amylase producing fungi. Maximum starch hydrolysis was recorded in 6 to 7 days old culture for both *A. fumigatus* and *C. globosum*. However,  $\alpha$ -amylase activity was found higher in 9 to 11 days old culture. Starch hydrolysis,  $\alpha$ -amylase and protease activity of *A. fumigatus* was found higher as compare to *C. globosum*. At higher temperature (45°C) also performance of *A. fumigatus* was better than *C. globosum*. As *Aspergillus fumigatus* was found to be better thermotolerant, it can therefore be utilize in starch processing industry as well as for amylase production at high temperature level. (*Asian Journal of Biochemistry* 3 (5): 284-289, 2008; *doi*: 10.3923/ajb.2009.284.289)



## **Kinetic and Mechanism Studies of the Reaction Between L-Tyrosine and Iodine on the Basis of UV-Vis Spectrophotometric Method**

M. Aghaie, M. Mirzaie, K. Zare, M. Monajjemi and H. Aghaie

This study concern to the kinetic and mechanism studies of interaction between L-tyrosine (L-T) and iodine upon UV-Vis spectrophotometric measurements. The results showed that the reaction is first order respect to each reactant by using the initial rate method. Also, the rate constant of the reaction at two different temperature 25.0 and 15.0°C were determined. Then the resulting activation functions such as  $E_a$ ,  $\Delta H_{\#}$ ,  $\Delta G_{\#}$ ,  $\Delta S_{\#}$  and frequency factor,  $A$ , were calculated. The resulted values at 25.0°C are:  $E_a = 36.82 \text{ kJ mol}^{-1}$ ,  $A = 2.032 \times 10^6 \text{ L mol}^{-1} \text{ sec}^{-1}$ ,  $\Delta G_{\#}^{\circ} = 73.17 \text{ kJ mol}^{-1}$ ,  $\Delta H_{\#}^{\circ} = 34.34 \text{ kJ mol}^{-1}$  and  $\Delta S_{\#}^{\circ} = -132.4 \text{ J mol}^{-1} \text{ K}^{-1}$ . (*Asian Journal of Biochemistry* 3 (5): 290-296, 2008; **doi**: 10.3923/ajb.2009.290.296)

## **Process Parameters Optimisation of Mannanase Production from *Aspergillus niger* FTCC 5003 Using Palm Kernel Cake as Carbon Source**

S. Abd-Aziz, L.G.A. Ong, M.A. Hassan and M.I.A. Karim

The aims of this study are to optimised the process parameters involved for submerged fermentation of mannanase by *Aspergillus niger* FTC5003 using palm kernel cake (PKC) as carbon source. The parameters investigated include temperature, agitation speed, inoculum size and PKC concentration. The submerged fermentation was carried out for 10 days by using palm kernel cake as the sole carbon source with the addition of nitrogen source. Palm kernel cake can be considered as a suitable carbon source for the enzyme production due to it mainly consist of mannan and galactomannan, which is hemicellulose. The mannanase enzyme production using the optimised fermentation condition, which was conducted at agitation speed of 200 rpm, temperature 35°C,  $1 \times 10^4$  spores  $\text{mL}^{-1}$  of inoculum size and 2% of palm kernel cake with productivity of 13.00 U/mL/day. (*Asian Journal of Biochemistry* 3 (5): 297-307, 2008; **doi**: 10.3923/ajb.2009.297.307)

## **Hepatoprotective Activity of Bi-Herbal Ethanolic Extract on CCl<sub>4</sub> Induced Hepatic Damage in Rats**

P. Samudram, H. Rajeshwari, R. Vasuki, A. Geetha and P. Sathiya Moorthi

The combined hepatoprotective effect of Bi-Herbal Ethanolic Extract (BHEE) pretreatment against carbon tetra chloride (CCl<sub>4</sub>) induced hepatic damage in rats was investigated. Ethanolic extract from the leaves of *Melia azedarach* and seeds of *Piper longum* at a dose level of 50 mg kg<sup>-1</sup> body weight was administered orally daily once for 14 days, prior to the administration of 0.2 mL of CCl<sub>4</sub> kg<sup>-1</sup> body weight for 7 days. The substantially elevated serum marker enzymes such as Serum glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Lactatedehydrogenase (LDH), Gamma glutamyl Transferase (γGT) and 5' Nucleotidase (5'NT), due to CCl<sub>4</sub> treatment were restored towards normalization in rats pretreated with BHEE. The determination of liver weight and biochemical parameters such as serum bilirubin and blood urea showed a remarkable decrease in rats pre-treated with the BHEE when compared with those administered CCl<sub>4</sub> alone. In addition BHEE significantly increased the total protein, total cholesterol and triglyceride also towards normal levels. Silymarin at a dose level of 50 mg kg<sup>-1</sup> was used as a standard reference also exhibited significant hepatoprotective activity against CCl<sub>4</sub> induced hepatotoxicity. The results of this study strongly indicate that BHEE has got a potent hepatoprotective action against CCl<sub>4</sub> induced hepatic damage in rats. (*Asian Journal of Biochemistry* 3 (5): 308-314, 2008; **doi**: 10.3923/ajb.2009.308.314)

## **Lipid Peroxidation and Total Antioxidant Capacity in Patients with Chronic Renal Failure**

D.R. Suresh, C.R. Wilma Delphine Silvia and Rajni Agarwal

This study was carried out to assess the status and correlation between lipid peroxidation and antioxidant status in CRF patients. Forty predialytic CRF patients, of either sex and age >25 years with serum creatinine levels >3.0 mg dL<sup>-1</sup> and forty healthy controls were included in the study. Serum MDA levels were estimated by colorimetric method and Total Antioxidant Capacity (TAC) was estimated by Ferric Reducing Antioxidant Power (FRAP) assay. It was observed that statistically significant increase in MDA levels in CRF patients correlating positively with creatinine and BUN and significant decrease in TAC in CRF patients correlating negatively with creatinine and BUN. Significant negative

correlation was observed between MDA and TAC in CRF patients which is suggestive of increased lipid peroxidation and depletion of antioxidants causing an imbalance between OFR and TAC leading to progressive renal injury. This study would be beneficial to identify patients with increased risk of CRF and also for monitoring and optimization of antioxidant therapy. (*Asian Journal of Biochemistry* 3 (5): 315-319, 2008; doi: 10.3923/ajb.2009.315.319)

### **Identification of Catalytically Essential Amino Acid Residues and Immobilization of Glutamate Dehydrogenase from *Rumex* Cotyledons**

H.M. El-Shora and M.M. Youssef

The present study was designed to characterize and immobilize the glutamate dehydrogenase (GDH, EC 1.4.1.2) from *Rumex dentatus* cotyledons. The enzyme from *Rumex dentatus* was purified with specific activity of 145 U mg<sup>-1</sup> protein. The indispensable role of arginine, lysine and tyrosine at the active site of the enzyme was demonstrated through chemical modification by 1, 2-cyclohexanedione (CHD), trinitrobenzenesulfonic acid (TNBS) and tetranitromethane (TNM), respectively. The three modifiers were inactivated GDH enzyme with pseudo-first order kinetics and second order-rate constants of 22, 0.70 and 0.5 mM<sup>-1</sup> min<sup>-1</sup>, respectively. Both  $\alpha$ -ketoglutarate and NADH offered GDH a protection against the inactivation. These studies suggested the involvement of arginine, lysine and tyrosine residues in the enzyme catalysis. GDH immobilized on gelatin beads via cross-linking with glutaraldehyde. The resulting immobilized enzyme was stored at 4°C for 10 days without losing its activity. K<sub>m</sub> of immobilized enzyme increased while V<sub>max</sub> reduced compared to the free one. The immobilization of GDH resulted in a shift of pH optimum from 7.5 to 8.0. The optimum temperatures of the free and immobilized enzyme were 45°C and 60°C, respectively. The immobilized enzyme has a long life as compared with the native enzyme. (*Asian Journal of Biochemistry* 3 (5): 320-329, 2008; doi: 10.3923/ajb.2009.320.329)

### **Rationalization of Physicochemical Property of Some Substituted Benzimidazole Bearing Acidic Heterocyclic Towards Angiotensin II Antagonist: A QSAR Approach**

Anurekha Jain and S.C. Chaturvedi

Various lead structures of the compounds of angiotensin II antagonist are reported in the literature. Studying the Structure-Activity Relationships (SAR) for such

compounds has been a fascination for scientists and efforts have been made to identify the essential physico-chemical requirements for the angiotensin type 1 (AT1) receptor selective, angiotensin type 2 (AT2) receptor selective and some AT1 and AT2 balanced antagonistic activity compounds. With an aim to identify the structural requirements for selective AT1 activity, a Quantitative SAR (QSAR) analysis was carried out on a series of benzimidazole derivative bearing acidic heterocycle AII receptor antagonists. The QSAR expressions were generated using 19 compounds and the predictive ability of the resulting model was evaluated against a test set of 7 compounds. The internal (cross validated squared correlation coefficient) and external consistency (predictive correlation coefficient) of the QSAR model was 0.895 and 0.405, respectively. Analysis of result from the present QSAR study indicates that geometrical, structural and shape descriptors govern the angiotensin II AT1 inhibitory activity. (*Asian Journal of Biochemistry* 3 (5): 330-336, 2008; *doi*: 10.3923/ajb.2009.330.336)

### **Cloning, Purification, Characterization and Immobilization of L-asparaginase II from *E. coli* W3110**

Magdy M. Youssef and Mohammed A. Al-Omair

In the present study, we report the cloning of L-asparaginase II (*asnII*) gene from *E. coli* W3110 into pGEX-2T DNA vector. The L-asparaginase II enzyme (E.C.3.5.1.1) was overexpressed in *E. coli* BL21(DE3) and purified to homogeneity 238.4 fold by utilizing chromatography technique on DEAE-Sepharose fast flow, Glutathione S sepharose 4B columns and thrombin. SDS-PAGE of the purified enzyme revealed that has *Mr* of 40 kDa. In addition, we found that the enzyme can be efficiently immobilized in calcium alginate gelatin composites. The free enzyme has an optimum pH at 7.5 but this optimum pH is shifted to 8.5 for the immobilized enzyme. The optimum temperature, for free and immobilized enzyme were 37 and 50°C, respectively. The immobilized enzyme retained most of its activity at 60°C with high stability compared with the native enzyme when incubated at 60°C for 30 min. (*Asian Journal of Biochemistry* 3 (6): 337-350, 2008; *doi*: 10.3923/ajb.2009.337.350)

### **Mutagenesis of Arginine-186 Located on a Helix Interacting with Pyridoxal 5'-Phosphate in *O*-Acetylserine Sulphydrylase**

S.I. Ozaki and M. Yoshiya

We mutated Arg-186, which was located in the middle of a helix interacting with the phosphate group of PLP in OASS, to study effects on catalytic properties. The

Arg-186 to Pro mutation completely inactivated the enzyme due to a loss of PLP. In contrast, R186L OASS retained one PLP molecule per subunit. The replacement of Arg-186 with Leu accelerated the reaction with OAS by 1.8-fold and the aminoacrylate intermediate of R186L OASS was converted to the internal Schiff base with azide or thiosulfate faster than that of the wild type by 1.5 and 1.3-fold, respectively. Although the changes in catalytic properties were moderate, the loss of guanidino group might have resulted in the reorganization of active site structure. (*Asian Journal of Biochemistry* 3 (6): 351-358, 2008; **doi**: 10.3923/ajb.2009.351.358)

### **Evaluation of Pesticide and Heavy Metal Toxicity Using Immobilized Enzyme Alkaline Phosphatase with an Electrochemical Biosensor**

L.K. Shyuan, L.Y. Heng, M. Ahmad, S.A. Aziz and Z. Ishak

An bioelectrochemical sensor or biosensor based on the inhibition of the enzyme Alkaline Phosphatase (ALP) has been investigated for the screening of several environmental toxicants. The biosensor was constructed by immobilizing ALP in a hybrid sol-gel/chitosan film that was deposited on the surface of a screen-printed carbon paste electrode (SPE). The inhibition was measured via the catalytic hydrolysis of ascorbic acid 2-phosphate (AA2P) by the enzyme to produce ascorbic acid. Oxidation of this product was monitored amperometrically and the current change was then related to ALP activity. Toxicity of herbicides (2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), insecticides (carbofuran and  $\alpha$ -endosulfan) and heavy metals ( $\text{Hg}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ag}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$ ) towards the biosensor were evaluated. Various degrees of inhibition of ALP occurred when the biosensor was exposed to herbicides and heavy metals. This resulted in a lower acid ascorbic production by the enzyme from the substrate, thus a decrease in the current response of the biosensor. The herbicides 2,4-D and 2,4,5-T showed the largest inhibition effect on ALP with linear response range of 1-60  $\mu\text{g L}^{-1}$  ( $R^2 = 0.92$ ). The maximum inhibitions caused by 2,4-D and 2,4,5-T were 46 and 30%, respectively. Heavy metals caused inhibition on ALP at the higher concentration range of  $\text{mg L}^{-1}$ . Thus, the biosensor may be useful for the screening of chlorophenoxyacetic acid herbicides even in the presence of other environmental toxicants. (*Asian Journal of Biochemistry* 3 (6): 359-365, 2008; **doi**: 10.3923/ajb.2009.359.365)

## **Biochemical, Hematological and Histopathological Effects of *Duranta repens* Stems on Rats**

F. Nikkon, S. Hasan, M.H. Rahman, M.A. Hoque, A. Mosaddik and M.E. Haque

Two triterpenes,  $\beta$ -Amyrin and 12-Oleanene 3 $\beta$ , 21 $\beta$ -diol, were isolated as a mixture from the chloroform soluble fraction of an ethanolic extract of *Duranta repens* stem. The structures of these two compounds were confirmed by IR,  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectral data. In acute toxicity test, the chloroform soluble fraction showed toxic effect on rats but compound 1 (mixture of  $\beta$ -Amyrin and 12-Oleanene 3 $\beta$ , 21 $\beta$ -diol) had no toxic effect on rat at 2 mg/kg/day b.wt. Biochemical and hematological profiles of rat's blood of chloroform soluble fraction were found statistically significant. In histopathological experiment, some degenerative changes on cellular structure of liver, heart and kidney except lungs were found in chloroform soluble fraction treated rats. (*Asian Journal of Biochemistry* 3 (6): 366-372, 2008; doi: 10.3923/ajb.2009.366.372)

## **Mineral Contents of Milk of Cattle, Camels, Goats and Sheep in the Central Region of Saudi Arabia**

N.A. Al-Wabel

Milk samples were obtained during the second month of lactation from cattle, camels, sheep and goats for analysis of their essential mineral content. The Mean $\pm$ SD concentrations of Zn, Mn, Cu, Fe, Ca, Na and K in the milk of cattle were calculated as mg kg $^{-1}$  on dry matter basis and were as follows: 2.0 $\pm$ 0.28, 1.291 $\pm$ 2.43, 1.80 $\pm$ 1.10, 4.214 $\pm$ 1.78, 661.0 $\pm$ 41.95, 91.6 $\pm$ 3.45 and 113.7 $\pm$ 5.84, respectively; for camels: 1.48 $\pm$ 0.76, 1.299 $\pm$ 0.11, 1.61 $\pm$ 0.90, 2.981 $\pm$ 2.24, 699.3 $\pm$ 96.65, 115.87 $\pm$ 4.99 and 133.77 $\pm$ 5.64, respectively; for goats: 1.129 $\pm$ 0.04, 0.57 $\pm$ 0.20, 4.908 $\pm$ 2.66, 751.72 $\pm$ 72.78, 101.3 $\pm$ 10.71 and 123.85 $\pm$ 9.94, respectively; for sheep: 3.09 $\pm$ 0.91, 1.144 $\pm$ 0.05, 0.62 $\pm$ 0.22, 5.011 $\pm$ 3.24, 822.5 $\pm$ 113.36, 95.4 $\pm$ 5.47 and 127.4 $\pm$ 11.10, respectively. Milk obtained from the species of animals investigated above was free from lead and cadmium. (*Asian Journal of Biochemistry* 3 (6): 373-375, 2008; doi: 10.3923/ajb.2009.373.375)

## **Territorial Investigation Based on the Chemical Composition of Chemlali Virgin Olive Oils**

D. Krichene, A. Allalout, B. Baccouri, G.Q. Fregapane, M.D. Salvador and M. Zarrouk

The purpose of this study was to evaluate differences in the chemical composition of virgin olive oils from the Chemlali variety cultivated in different olive growing areas of the Centre of Tunisia. All samples were harvested using the same controlled procedures and were submitted to a controlled processing in the same laboratory mill. Several analytical parameters and indices were determined. Results showed that the oils quality was attributed not only to the olive variety but also to the plantation site, therefore to climatic and pedologic factors. All these parameters showed an important effect on the fatty acid, phenol,  $\alpha$ -tocopherol, sterol and volatile contents of the oils. (*Asian Journal of Biochemistry* 4 (1): 1-12, 2009; **doi**: 10.3923/ajb.2009.1.12)

### **Nephrotoxicity Reduction by Fixed Dose Combination of Cephalosporins and Aminoglycosides in *Mus musculus* Mice**

V.K. Dwivedi, M. Chaudhary, A. Soni and S.M. Shrivastava

Free radicals are causative factors for aminoglycoside induced renal toxicity. The aim of present study was to evaluate effect of fixed dose combination of cefepime+amikacin (Potentox) as well as ceftazidime+tobramycin (Tobrcef) antibiotics on antioxidant enzymes (Super oxide dismutase, Catalase and Glutathione reductase) along with (free radical mediated damage) malonaldehyde levels and extracellular antioxidant enzymes (creatinine, total bilirubin and uric acid enzymes) in kidney tissue of *Mus musculus* mice. Present findings showed that the activities of the antioxidant enzymes were significantly lowered along with increase in MDA (malonaldehyde) levels and extracellular antioxidants after single treatment of aminoglycosides (amikacin and tobramycin) as compared to control group. A significant improvement in antioxidant enzymes along with significant decrease in creatinine, total bilirubin, uric acid and malonaldehyde (MDA) levels were observed in fixed dose combination of cefepime plus amikacin as well as ceftazidime+tobramycin treated groups compared to amikacin and tobramycin alone treated group. These results indicate that a fixed dose combination of cephalosporins with aminoglycosides using chemical vector mediated technology acts as an antioxidant and prevents nephrotoxicity induced by aminoglycosides. (*Asian Journal of Biochemistry* 4 (1): 13-21, 2009; **doi**: 10.3923/ajb.2009.13.21)

## **Selenium and $\alpha$ -Difluoromethylornithine in Combination have Strong Activity Against Elevated Polyamines and Glucose Levels in Serum**

Mohammed A. Al-Omair

In the present study mice were supplemented with 2% of both L-arginine and L-ornithine in drinking water for four weeks. L-arginine and L-ornithine intake elevate polyamine levels in serum of female Swiss albino mice. The effect of selenium (Se) administration (as sodium selenite: 0.5 or 1 mg kg<sup>-1</sup> body weight) or/and  $\alpha$ -difluoromethylornithine (DFMO: 2 mg kg<sup>-1</sup> body weight) on the elevated polyamine levels was studied. The elevated polyamine levels were decreased significantly by administration of low and high doses of Se with DFMO. Glucose concentration in the serum increased significantly with high polyamine level of groups and reduced back around the normal values by Se and DFMO treatment. The concentrations of triglycerides and cholesterol are not effected by the elevated levels of polyamines in the serum. These results suggest that administration of Se in combination with DFMO protect cells from the harmful effect of high levels of polyamines. (*Asian Journal of Biochemistry* 4 (1): 22-29, 2009; doi: 10.3923/ajb.2009.22.29)

## **Impact of *Plasmodium berghei* and Chloroquine on Haematological and Antioxidants Indices in Mice**

H.O.T. Iyawe and A.O. Onigbinde

The effect of malaria parasites and chloroquine in mice was examined. The importance of this study derives from the prevalence of malaria in the tropical and subtropical regions, as well as the declining therapeutic efficacy of chloroquine as a first line treatment against malaria infection in these endemic areas. This study aimed to determine the pattern of possible alterations in some haematological and antioxidant molecules in mice treated with either Plasmodium or chloroquine. Three groups of ten mice each categorized as control, non parasitized chloroquine treated (NPcqT) and Parasitized non treated (PnT) were used in this study. Observations from the work show that parasites in mice significantly ( $p < 0.05$ ) increased plasma total protein, globulin, erythrocyte fragility, total bilirubin, oxidative stress, glucose-6-phosphate dehydrogenase (G6PD), liver superoxide dismutase (SOD) and catalase (CAT) enzyme activities. Also the study showed that there is a significant ( $p > 0.05$ ) decrease in plasma SOD, CAT, reduced glutathione (GSH), liver G6PD and GSH. Parasitemia also reduced significantly



( $p < 0.05$ ) mice packed cell volume (PCV). Chloroquine treatment of Non Parasitized (NP) mice increased significantly ( $p < 0.05$ ) erythrocyte fragility, plasma total bilirubin, oxidative stress, but reduced ( $p < 0.05$ ) mice PCV, plasma SOD, CAT, G6PD, GSH but increased ( $p < 0.05$ ) liver SOD, CAT and reduced GSH significantly ( $p < 0.05$ ). The results obtained from the statistical analysis of data suggest that both malaria parasites increase oxidative stress in mice and chloroquine increases SOD and CAT activity in hepatic tissue of mice. (*Asian Journal of Biochemistry* 4 (1): 30-35, 2009; doi: 10.3923/ajb.2009.30.35)

### **Molecular Existence of Mature Odontoblast and Osteoblast Cells in Adult Human Pulp Tissues**

A. Eni Juliana, Z.A. Shahrul Hisham, M.A.W. Rohaya and S. Nik Marzuki,

The dental pulp tissue is essential in dentine development. The existence of Dental Pulp Stem Cells (DPSCs), i.e., osteoblast and odontoblast are to assist in dentine repair and tooth regeneration. The existence of osteoblast that secreted bone matrix directly from pulp tissue has not been reported. The purpose of this study is to determine the existence of odontoblast and osteoblast cells excavated directly from pulp tissues by using molecular markers. The isolated RNA expressing two gene markers, i.e., dentin sialophosphoprotein and osteocalcin which were secreted by odontoblast and osteoblast cells, respectively. The expression of dentin sialophosphoprotein and osteocalcin demonstrated that both odontoblast and osteoblast cells exist in adult human pulp tissues. (*Asian Journal of Biochemistry* 4 (2): 36-44, 2009; doi: 10.3923/ajb.2009.36.44)

### **Study on Apparent Amylose Content in Context of Polymorphism Information Content along with Indices of Genetic Relationship Derived through SSR Markers in *Birain*, *Bora* and *Chokuwa* Groups of Traditional Glutinous Rice (*Oryza sativa* L.) of Assam**

B. Shaptadvipa and R.N. Sarma

Amylose content was determined in 41 traditional glutinous rice varieties of Assam classed as *Birain*, *Bora* and *Chokuwa* group during 2004-06. Average apparent amylose content in 6 accessions of *Chokuwa* (9.368%) was higher than 20 accessions of *Bora* group (0.502%) and 15 accessions of *Birain* (0.191%) genotypes. *Mahsuri*, a non-glutinous rice variety contained intermediate amylose content (21.2%). Eight SSR markers were used to assess genetic variability. The

size of amplified fragments ranged from 100 to 500 bp. Among all genotypes, average Polymorphism Information Content (PIC) was 0.923. The average genetic similarity within the *Birain* accessions ranged from 0.119 to 0.571. Within *Bora-Chokuwa* accessions, similarity value ranged from 0.047 to 0.667. The average similarity was 0.228, which reflected that the *Bora* group could be more diverse than the *Birain* group. Amylose content is said to be highly influenced by environmental conditions. Since, *Birain* accessions were from the same Barak valley agro-climatic condition and *Bora* as well as *Chokuwa* were from the Brahmaputra valley, an analysis was made with corresponding pair-wise relative rate of increase (%) in apparent amylose contents as well as corresponding values of pair-wise Jaccard's co-efficient of similarity among the accessions of *Birain*, *Bora* and *Chokuwa* groups of glutinous rice. It showed the existence of a matching relation between the increased values of respective apparent amylose content and the genetic similarity. It seems that apparent amylose content though cannot play a solid indicator for genetic variability in glutinous rice germplasm. However it may help to gauge biochemical bases towards genetic variability under same environmental condition. (*Asian Journal of Biochemistry*, 4 (2): 45-54, 2009; doi: 10.3923/ajb.2009.45.54)

### **Effect of Artemisinin with Folic Acid on the Activities of Aspartate Amino Transferase, Alanine Amino Transferase and Alkaline Phosphatase in Rat**

Aniefiok Udobre, Edoho J. Edoho, Olorunfemi Eseyin and Emmanuel I. Etim

Studies on the effect of artemisinin alone and artemisinin with folic acid on the activities of aspartate amino transferase (ASAT), alanine amino transferase (ALAT) and alkaline phosphatase (ALP) in the serum of male Wistar rats were carried out. Different groups of rats (8 group<sup>-1</sup>) were orally given 0.75, 1.50, 3.00 and 6.00 mg kg<sup>-1</sup> b.wt. of artemisinin. Each of these doses was also administered concurrently with 1.50 mg kg<sup>-1</sup> of folic acid, respectively. Artemisinin only elevated the activities of serum ASAT, ALAT and ALP significantly at the four dose levels. When 1.50 mg kg<sup>-1</sup> of folic acid was concurrently administered with artemisinin, the elevated serum level of ASAT, ALAT and ALP was significantly reversed almost completely at low dose of 0.75 and 1.50 mg kg<sup>-1</sup> artemisinin. Folic acid only reversed the elevated activity of ASAT, ALAT and ALP by artemisinin partially when the dose of artemisinin was high (i.e., 3.00 and 6.00 mg kg<sup>-1</sup> of artemisinin). These results suggest that folic acid offers complete relief to metabolic disorders at low artemisinin concentration while the relief is partial at high concentrations. (*Asian Journal of Biochemistry*, 4 (2): 55-59, 2009; doi: 10.3923/ajb.2009.55.59)

## ***Nigella sativa* Modulates Cytokines Expression in Mature Bovine Adipocytes**

M.M. Soliman, Y.A. El-Fattah El-Senosi, O.M.A. El-Hamid, A.El-Desouki Abd El-Mageed, R.S. Ismaeil and H.A. El-Maqsoud Ali

In this study, we examined the effect of either lipopolysaccharide (LPS,  $1 \mu\text{g mL}^{-1}$ ), *Nigella.sativa* extract (NS,  $5 \mu\text{g mL}^{-1}$ ), or co-treatment of both for 24 h on cytokines expression in mature bovine adipocytes using RT-PCR analysis. The results showed that separate treatment by LPS and NS stimulated the expression of interleukin-1 (IL-1 $\beta$ ), IL-6, IL-8 and IL-10. Co-treatment of cells by *N. sativa* with LPS inhibited LPS induced IL-6 and TNF- $\alpha$  expression and induced additive stimulatory effect on LPS induced IL-8 and IL-10 expression. The results indicate that *N. sativa* extract has immuno-modulatory effect on bovine adipocytes by stimulating different cytokines expression that potentiate different inflammatory and anti-inflammatory functions in bovine adipocytes. (*Asian Journal of Biochemistry*, 4 (2): 60-67, 2009; doi: 10.3923/ajb.2009.60.67)

## **Fermented Soybean Products: Some Methods, Antioxidants Compound Extraction and their Scavenging Activity**

I. Amadou, S. Yong-Hui, J. Sun and L. Guo-Wei

Antioxidant compounds in food such as phenolic compounds played various roles as health promoting factors (e.g., cancer and cardiovascular disease), antimicrobial agents; flavor active compounds, colorants precursors and colloidal stability affecting factors as well as chelating agents. Fermented foods such as soy products have been in existence for thousands of years and received attention as sources of many effective antioxidants. This review discusses about the most commonly used fermented soybean food antioxidants, methods of their preparation and description of their scavenging activity/antiradical property. (*Asian Journal of Biochemistry* 4 (3): 68-76, 2009; doi: 10.3923/ajb.2009.68.76)

## **Characterization of a *Capsicum chinense* Seed Peptide Fraction with Broad Antibacterial Activity**

L. Brito-Argáez, F. Moguel-Salazar, F. Zamudio, T. González-Estrada and I. Islas-Flores

Habanero chili pepper (*Capsicum chinense*) is widely consumed as a fresh vegetable, although its extremely high capsaicin content has led to other uses (e.g.,

medicine and self-defense). Recently described antimicrobial peptides from *C. annuum* were very efficient in inhibiting growth in human and plant pathogenic bacteria and fungi. In order to explore the potential use of *Capsicum chinense* seeds as a source of antimicrobial peptides, in the present study a peptide fraction from *C. chinense* pepper seeds, denominated G10P1, was enriched, partially purified and its antimicrobial activity tested against the plant and human pathogens *Xanthomonas campestris*, *Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Erwinia carotovora*, *Agrobacterium* sp., *Shigella flexnerii*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The minimum inhibitory concentration of the G10P1 peptide fraction against *X. campestris* was 12.5 µg mL<sup>-1</sup>. Electrophoresis of the G10P1 in a denaturant 15% polyacrylamide gel showed it to be composed of ~7.57 and ~5.6 kDa polypeptides, both associated with an area of strong antibacterial activity. The sequencing of 18 amino acids from the N-terminal of the ~7.57 peptides and 12 from the ~5.6 kDa peptides showed no clear association with previously described antimicrobial peptides. However, the ~5.6 kDa peptides were related to the NAC and WRKY transcription factors, both involved in direct regulation of the plant defense response against pathogen attack and the ~7.57 kDa peptides had low homology with a 3-oxo-[acyl-carrier-protein] synthase from *Capsicum chinense*. (*Asian Journal of Biochemistry* 4 (3): 77-87, 2009; doi: 10.3923/ajb.2009.77.87)

### **Extraction, Characterization and Nutritional Properties of Two Varieties of Defatted Foxtail Millet Flour (*Setaria italica* L.) grown in China**

M.T. Kamara, Z.H. Ming and Z. Kexue

In this study, we examined the various protein fractions and protein concentrates of two selected varieties (white and yellow) of foxtail millet grown in China. Characterized by amino acid analysis, differential scanning calorimetry (DSC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein content for the white increased after defatting though not significantly different ( $p < 0.05$ ) from 11.50 to 11.59 and the yellow decreased from 11.41 to 11.39. Fat content shows a significant decreased ( $p < 0.05$ ) after defatting from 2.38 to 0.41 (white) and 2.90 to 0.66 (yellow). Prolamin yellow and glutelin white were the major fractions (38.8 and 47.2%, respectively), followed by albumin yellow and white as 2.6 and 1.5%, respectively and globulin yellow and white as 2.5 and 1.4%, respectively and the difference was significant ( $p < 0.05$ ) among the various protein fractions. Results showed a significant amount of amino acids with essential amino acids above the recommended amount by FAO/WHO for

humans. Albumin white possessed the highest DSC result ( $T_p = 79.583^\circ\text{C}$ ,  $\Delta H = 5.115 \text{ J g}^{-1}$ ), glutelin white the lowest ( $T_p = 66.682^\circ\text{C}$ ,  $\Delta H = 0.313 \text{ J g}^{-1}$ ). Fractions and concentrates had molecular sizes below 14.0 and above 97.0 kDa. Protein fractions and concentrates are potential as functional food ingredient. (*Asian Journal of Biochemistry* 4 (3): 88-98, 2009; *doi*: 10.3923/ajb.2009.88.98)

### **Influence of Cultural Conditions on Glutathione Peroxidase Synthesis in *Candida albicans***

R. Yang, N. Mohan, C. Muthukumar, N. Thajuddin and M. Gunasekaran

The influence of cultural conditions that affect GPX production in *Candida albicans* grown in Lee's medium was investigated. Optimum temperature and pH for GPX activity were  $25^\circ\text{C}$  and 7.2, respectively. Substrate specificity for *C. albicans*. Glutathione peroxidase was in the order of cumene hydroperoxide > t-butyl hydroperoxide > hydrogen peroxide > benzoyl peroxide. Aeration as well as large head space volume enhanced the growth of *C. albicans* and GPX production. Arabinose and ammonium sulphate significantly increased the GPX synthesis. Among nitrogen sources, polypeptone enhanced both the growth and GPX synthesis. Various cellular activities are regulated by the level of GSH. Therefore, the level of GPX might be used as one of the criteria in developing new drugs against *Candida albicans*. (*Asian Journal of Biochemistry* 4 (3): 99-105, 2009; *doi*: 10.3923/ajb.2009.99.105)

### **Entropy Driven Binding of O-Glycan and Glycoproteins to *Artocarpus hirsuta* Lectin: An SPR Study**

F. Khan, S.M. Gaikwad and M.I. Khan

In this study, thermodynamics of binding of O-glycan (Gal $\beta$ 1-3GalNAc $\alpha$ 1-O-Ser) and the glycoproteins possessing it, viz., fetuin and mucin to *A. hirsuta* lectin was studied using Surface Plasmon Resonance (SPR). The binding affinities were in the order of asialomucin > mucin > asialofetuin > fetuin > O-glycan and found to increase with increase in valency of the ligand. Unusual for a lectin-ligand interaction, the binding was endothermic and entropically driven and the higher affinity was associated with a large favorable entropy term. The native fetuin and mucin showed lower affinity than their desialylated counterpart. Kinetic analysis of the binding revealed that the difference in the affinity of different ligands was due to different rates of their association, whereas the dissociation rates were similar and

showed decrease with temperature. The activation energy of the association process was lower with desialylated glycoproteins than that of sialylated one resulting in their faster association and higher affinity. (*Asian Journal of Biochemistry* 4 (4): 106-116, 2009; doi: 10.3923/ajb.2009.106.116)

### **Mutagenesis of Gln-142 and Phe-143 of O-Acetylserine Sulfhydrylase**

S. Ozaki, A. Nakahara and C. Sakaguchi

In order to examine the substrate-binding site of *O*-acetylserine sulfhydrylase (OASS) from *Escherichia coli* (*E. coli*), we mutated Gln-142 and Phe-143, which exist at a  $\beta$ -turn region in the active site. The mutants retained one molecule of pyridoxal 5'-phosphate (PLP) per subunit and PLP was covalently bound in Schiff base linkage, similar to what was observed for the wild type enzyme. Q142A and F143Y OASS inhibited the reaction with *O*-acetylserine and the subsequent formation of the amino acrylate intermediate. The F143A, S and D mutants were able to form the amino acrylate intermediate, but the rate was significantly slower than that of the wild type enzyme. These results suggest that mutagenesis of Gln-142 and Phe-143 residues in OASS influence catalytic properties, possibly due to modulation of the substrate-binding site. (*Asian Journal of Biochemistry* 4 (4): 117-124, 2009; doi: 10.3923/ajb.2009.117.124)

### **Biochemical and Histological Changes Associated with Long Term Consumption of *Gnetum africanum* Welw. Leaves in Rats**

Emeka E.J. Iweala, Friday O. Uhegbu and O. Obidoa

Changes in some biochemical and haematological indices including serum protein, haemoglobin, cholesterol, lipid peroxidation, white blood cells, Glutathione-s-transferase, Superoxide dismutase, Alanine transaminase, Aspartate transaminase and Alkaline phosphatase were investigated in male rats fed with a diet supplemented with leaves of *Gnetum africanum*. The histological changes on the liver, intestines and testes were also examined. The long term feeding of the *Gnetum africanum*-supplemented diet caused significant increases ( $p < 0.05$ ) in weight, haemoglobin and white blood cells. There were also significant increases ( $p < 0.05$ ) in Glutathione-s-transferase and Superoxide dismutase enzymes. However, *Gnetum africanum*-supplemented diet caused a significant reduction ( $p < 0.05$ ) in serum protein and lipid peroxidation. The liver enzymes namely

Alanine transaminase, Aspartate transaminase and Alkaline phosphatase were unaffected while the reduction in cholesterol was not significant. Histologically, the liver hepatocytes and hepatic plates were respectively elongated and enlarged while the intestinal mucosa showed elongated villi and enlarged submucosa. There were however no histological changes on the testes. (*Asian Journal of Biochemistry* 4 (4): 125-132, 2009; doi: 10.3923/ajb.2009.125.132)

### **Protective Effect of Squalene on Endogenous Antioxidant Vitamins in Experimentally Induced Myocardial Infarction in Rats**

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In the present study an attempt has been made to assess the cardioprotective effect of squalene on isoprenaline-induced myocardial infarction in male albino rats with respect to changes in the levels of endogenous antioxidant vitamins in heart tissue. Levels of endogenous antioxidants such as ascorbic acid,  $\alpha$ -tocopherol and endogenous squalene content in heart tissue were determined. Significant ( $p < 0.001$ ) reduction was observed in the levels of ascorbic acid,  $\alpha$ -tocopherol and endogenous squalene content in the heart tissue of isoprenaline administered rats compared to normal control rats. It is worth noting that, the prior administration of squalene at 2% level along with feed for 45 days significantly ( $p < 0.001$ ) reduced the isoprenaline-induced decline in the levels of these vitamins and restored the membrane bound squalene content at near normal. The results of the present study indicates that the cardioprotective effect of squalene might be ascribable to its antioxidant property thereby sharing the responsibility of these antioxidant vitamins in counteraction of free radicals generated during isoprenaline-induced oxidative stress. (*Asian Journal of Biochemistry* 4 (4): 133-139, 2009; doi: 10.3923/ajb.2009.133.139)