interaction between drugs and BLG converted to an important subject for many works today.Oxaliplatin is the third generation analogue of cisplatin that show activity against colon cancer and has demonstrated antitumor activity in cisplatin resistant cell lines. Furthermore, oxaliplatin is the only platinum compound to be clinically effective in colorectal cancer and is therefore frequently used as first-line treatment, of primary tumours, and second-line treatment, for recurring tumours, against this malignancy.In present investigation, we study the interaction of oxaliplatin with BLG using AutoDock 4.2 software for docking and analysis of binding sites of this drug on milk carrier protein of BLG.So, we have investigated the types and places of interaction which occurred between oxaliplatin and BLG. Our result shows that physical interactions such as electrostatic and hydrogen bonds have effective role in this interaction, then, BLG can be consider as a carrier of oxaliplatin. Our results are helpful for designing of new targeted drugs with lower side effects for cancer treatment.

Keywords: Oxaliplatin, BLG, Theoretical Method, AutoDock.

Abstract No.65

cDNA Cloning of IP3-Receptor Binding Core and Expression Study in E.Coli

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Inositol Trisphosphate (IP3) is a ubiquitous second messenger in eukaryotic cells that triggers Ca^{2+}-release from intracellular stores. IP3 binds to an intracellular receptor (IP3R) and induces conformational change within the ligand-binding domain which regulates Ca^{2+} release, hence, both IP3 and its receptor (IP3R) are key components of the signal transduction mechanism. Here we present cDNA cloning of IP3-binding core encoding only residues 224-604 of human IP3R type 2 that binds to IP3 with high affinity. RNA extraction (from fresh human spleen), RT-PCR, PCR and cloning were carried out, and then the cloned DNA was checked by sequencing. In order to test ligand-induced conformational change in IP3-binding core, the recombinant protein containing IP3 binding site is need. Therefore, the vector harboring of IP3-binding core was transformed to E.Coli BL21(DE3) to expression study in prokaryote system and characterized in the absence and presence of its ligand.

Keywords: Inositol 1,4,5-trisphosphate, Cloning , RT-PCR.

Abstract No.66

Threonine-Serine Protein Kinase B-Mediated Cytoprotection by White Radish (Raphanus sativus Linn.) Aqueous Extract on Lidocaine-Induced Neonatal Fibroblast Injury In Vitro

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Lidocaine as a classic amide-type local anesthetic is extensively utilized for epidural anesthesia. Several reports state that administration of lidocaine may lead to neuronal injury both locally and systemically in certain extreme cases, especially during birth, affecting not only the mother but also the newborn. Recently, there are also findings showing cytotoxicity in fibroblasts in vitro simulating local administration of the anesthetic and its toxicological effects in tissues at the point of entry. Raphanus sativus crude aqueous extracts (RSAE) have been analyzed to contain high concentrations of glucosinolates and isothiocyanates which have been extensively studied to promote cytoprotection against cellular stress induced either physically or chemically. In this study, the effects of RSAE were evaluated on lidocaine-induced cytotoxicity in human dermal neonatal fibroblasts (HDFn). HDFn were exposed to lidocaine in the presence or absence of RSAE. After exposure, cell viability and lactate dehydrogenase activity were evaluated including the mitochondrial transmembrane integrity. Relative quantitation of cfos and cjun expression as early markers of apoptosis was also determined. The cytoprotective capability of RSAE in preventing lidocaine-induced injury was also investigated. Exposure of HDFn cells to lidocaine resulted to significant cell injury confirmed by cytopathic effects, LDH leakage and upregulation of cfos and cjun. However, pre-treatment of the cells with RSAE significantly attenuate lidocaine-induced cell injury by stabilizing the mitochondrial potential with marked increase in Akt (threonine-serine protein kinase B)

phosporylation levels. Supportive evidence suggests that molecular inhibition of Akt by the benzimidazole-derived inhibitor IV totally rendered the cytoprotection by RSAE ineffective on lidocaine-induced cell injury. These findings infer the potential capability of natural plant extracts from radish in exerting a cytoprotective effect on lidocaine-induced injury in neonatal fibroblasts through the Akt signal transduction pathway.

Keywords: Akt, Cytoprotection, Anesthetic, Cell Injury.

Abstract No.67

Thermodynamics of Thermal Denaturation of Ribonuclease A in the Presence of Guanidine Hydrochloride and Urea

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Bovine pancreatic ribonuclease A (RNase A, E.C.3.1.27.1), one of the most extensively studied proteins,has 124 amino-acid residues and no tryptophan residues.Molecular weight of Ribonucleas A is 13 KD. The Second dominant structures consists of are the four long anti-parallel β strands and three short α Helix. Ribonucleas A catalyzes the cleavage of single-stranded RNA at the end of P-O5'pyrimidine nucleotides. Three residue Lys 41, His12 and His119 play an important role in catalysis by Ribonucleas A. Ribonucleas A is remarkably stable, and structurally and functionally very versatile.In this study,Tm of Ribonuclaes A as an indicator of thermal stability has been studied by UV-Vis spectrophotometry and spectrofluoremetry in the presence of Guanidine hydrochloride (GuHCl) and urea at pHs 1.5 and 3.3.The major conclusions based on our studyare that the thermal stability of ribonucleaseA decreases with increasing concentration of denaturants and that the effect of GuHCl on the thermal stability of RNase A is more pronounced than that of urea.GuHCl is decreased dielecteric constant of solvent more than urea.Urea can denature Ribonucleas A indirectly, By changing the structure and hydrodynamics of the solvent itself, similar to putting a non-polar solute into the mix, urea encourages the destabilization of internal bonds. It would then appear that direct interaction of urea with the protein, through hydrogen bonding, is the likely beginning of Ribonucleas A unraveling. By increasing Urea concentration from 0 to 4 M and Guanidine hydrochlorideconcentration from 0 to 3 M at pH=1.5 and 3.3 fluorescence emission is decreased.

Keywords: Bovine Pancreatic Ribonuclease A, guanidinine Hydrochloride (GuHCl).

Abstract No.68

Study on the Effect of Newly Synthesized Cateionic Pt (II)-complexes on Structure and Binding Properties of Human Serum Albumin

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Since human serum albumin (HSA) primarily serves as a transport carrier for steroids, fatty acids, thyroid hormones and variety of pharmaceutical drugs, it is important to study the interaction between this protein and potential drugs. Knowledge of the interaction mechanisms is of crucial importance to the understanding of the pharmacodynamics and pharmacokinetics of a drug. In the present study, the interactions between novel cationic Pt (II) complexes and HSA were investigated under different experimental setups, using fluorescence and circular dichroism (CD) instruments. Also as 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay performed to assess their inhibitory activity against growth of two leukemia cancer cell lines, range of anticancer activities were observed. Both florescence- and far-UV CD results suggest that Pt (II)-complexes can induce structural changes in HSA. Also the synthetic Pt (II)-complex revealed strong abilities to quench Trp fluorescence emissions of HSA. Moreover, thermodynamic parameters (ΔG, ΔH and ΔS) of the interaction between these complexes and HSA were calculated. The results revealed that the interaction was spontaneous, mainly entropy driven and primarily dominated with the hydrophobic forces. The results of current study clearly indicate that newly synthesized Pt (II)-complexes could effectively bind to HSA, inducing structural alteration in this protein.

Keywords: Cationic Platinum- (II) Complexes, Human Serum Albumin, Anticancer Activity, Fluorescence Study, Circular Dichroism.
