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Nerium oleander is a kind of medicinal plants and has different biological activity such as antibacterial,antioxidant and cytotoxic effects. In the present work we used this plant leaves extract for evaluation of teratogenic effects on chicken embryo,For investigation,at first, dichlorometan extract of the leaves preaperd. Dicholoromethan extract solutions in DMSO were injected in air sac of chicken eggs at concentrations of ,5,10,20,40,60 and 80 µg/eggs 72h after incubation recording of survival fraction of the chickens at 19th day was showed that 73.4, 66.7, 53.3, 46.7, 35.7and 26.6 percents of embryos were alive, respectively statistic analysis of the results showed that the extract induces mortality with LD50 of 24.80 µg/egg. The study of incidence of morphological and skeletal abnormality in the treatment groups showed that club foot, beak deformity and gastroschisis was occurred in morphology while caudal vertebrates deletion, unossification or uncalcification of caudal vertebrates and shortness of tibia were appeared in skeleton of the chickenes. These data imply that the lethal effect of extract against chicken embryo was occurred in a dose-dependent manner. The study of the embryotoxcity of dicholoromethan extract showed that, The leave extract of this plant can be used as a chemotherapy agent.

Keywords: Nerium Oleander, Toxicity, Abnormality, Chick Embryo.

Abstract No.80

Identification of Molecular Weight of Oocyte Vitellogenin Protein of Iranian Caviar in time of Inducing in Liver by Treatment of 17 Beta Estradiol Hormone, Secreted in to Bloodstream and Stored in Oocyte in Iranian Sturgeon as a Yolk Protein

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Iranian Caviar fish"Acipenser persicus" exist in the Caspian Sea basin (north of Iran). Unfortunately, nowadays, this fish has become an endangered species and listed as threatened, vulnerable, and endangered throughout their ranges. Population numbers of this fish has suffered a decline as a result of natural and anthropogenic factors such as the construction of dams, water pollution, over-fishing and commercial operations for Caviar production. This fish consume for Caviar production and good flesh quality in Iran. Main protein of Caviar is vitellogenin,a phospholipoglycoprotein which is synthesized in liver and secreted in to bloodstream for accumulation in oocyte in reproduction cycles. There is a little information on vitellogenin protein in Persian sturgeon fish. The aim of this study was to assess the size of vitellogenin protein in bloodstream, changes and cleavage during transportation into oocye in Persian sturgeon. Vitellogenin induction was detected after injection of 17 beta estradiol at concentration of at least 5 mg per kg of body weight. Immature sturgeons at juvenile stage do not naturally synthesize Vg, but strongly responded to exogenously injected hormone. After 3 injections, heparinated bloods were collected centrifuged in 4ºC and plasma was separated. Plasma was diluted and electrophored by SDS-PAGE method was showed molecular weight of vitellogenin in this species. Vtg is cleaved into three yolk protein components:lipovitellin, phosvitin and beta component. Beside injection of juvenile fish, oocyte protein was selected from ovulated fish and vitellogenin protein was purified. Serum of injected fish was dialysed against distilled water. The precipitate was separated by centrifugation at 10 000 g for 15min at 4ºC. The pellet was suspended in distilled water, recentrifuged and dissolved in 0.5 M Nacl. The solution was applied to a gel filtration column. Gel filteration with exact protein markers showed the size of this protein in blood. Gel preparation method was selected for purification of cleavaged Lipovitellin from vitellogenin in oocyte Antisera were raised in rabbits against purified vitellogenin in plasma and purified Lipovitellin(in oocyte) by intradermal injection of each sample emulsified in an equal volume of Freund complete adjuvant. Concentrations of proteins were 0.5 mg per kg body weight. Injections were conducted at four times. The Enzyme-linked immunosorbant assay for sturgeon vg was developed to quantify serum Vg, using purified sturgeon Vg and anti-Vg. At the end, this stage and western blotting were done for demonstrate induction of vitellogenin in blood.

Keywords: Acipenser Persicus, Phospholipoglycoprotein, Vitelogenin, Blood, Oocyte.

Abstract No.81

Stabilization of an Atypical Bacillus Amylase by Adding Extra DX unit in EF-hand Like Motif

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Calcium has a crucial role in activity and stability of amylases, all amylases have at least one Ca2+ per molecule, therefore specific and conserved amino acids are involved in calcium binding. In the present study, sequence analysis revealed the presence of EF-hand like motif in calcium binding loop of BMW-amylase that was previously isolated from Bacillus megaterium WHO. The EF-hand motif and its variants (EF hand like motif) are the most common calcium-binding motifs that found in various calcium binding proteins. We used site directed mutagenesis to replace His 58 with Asp (D), Ile (I), Tyr (Y), Phe (F) and Arg (R) at the seventh position of EF hand like motif to investigate the effect of calcium ion on the thermal stability, activity and structure of BMW-amylase. Effect of calcium on wild type and its variants, that monitored by fluorescence techniques, showed no significant changes. All mutants retained their hydrolytic activity and upon the addition of an extra DX unit to the calcium binding loop in H58D variant, thermal stability, catalytic activity and chelating power of the enzyme improved due to higher affinity toward calcium.

Keywords: EF-hand Like Motif, Calcium Binding Proteins, DX unit, BMW-Amylase, Chelating Power.

Abstract No.82

Evaluation of Metal-Ion Affinity Enrichment of Phosphoprotein

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Phosphorylation and dephosphorylation of cellular proteins play a fine tuning function during signal transduction that affect cellular processing and survival of living organism as a whole. Phosphoprotein profiling, thus, is an active research area to understand the molecular basic of cellular activities, but is hampered because of the low abundance of cellular phosphoproteins. Hence, enrichment of cellular phosphoprotein was highly recommended. Currently, three techniques: (I) immobilized metal ions affinity electrophoresis (IMAEP), (II) immobilized metal ion affinity chromatography (IMAC), and (III) immobilized metal ion affinity nanoscale magnetic beads (IMANMB) were used for phosphoprotein enrichment. These techniques share common principle, i.e., phosphoproteins are captured through interaction with metal ions that in turn is immobilized to inert matrix. Therefore, we have analyzed and compared the three techniques using purified phosphoproteins and cellular phosphoproteins. Comparison with IMAC (QIAGEN) shows that both IMAEP and IMANMB (Clontech) could be carried out with small sample size. In contrast, the enrichment duration was of following order: IMANMB < IMAC < IMAEP. The sensitivity with regard to sample size was nearly same between IMAEP and IMANMB but less in IMAC. However, using of lab synthesized IMANMB need to be undergoes thorough examination for its applicability compared with commercially available IMANMB. For example, in our recent case, IMANMB supplied by chemistry department, IASBS, shows the presence of non-phosphoprotein contaminants. This is not the case with Clontech IMANMB under similar condition of enrichment process. This contaminant seems to be mainly come from porosity and thickness of the outer coating layer of magnetic particles. The less removal of contaminants even after several washing with binding buffer and removal of the same along with the phosphoprotein with elution buffer clearly indicate that this non-specific binding is also electrostatically entrapped with either core/shell or central magnetic core.

Keywords: Phosphoprotein, Phospho Magnetic Beads, IMAEP, IMAC.

Abstract No.83

Bioinformatics Analysis of Helicobacter Pylori IceA, an Ulcer-associated Gene Restriction Endonuclease (nlaIIIR)

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Helicobacter pylori, which colonizes the gastric mucosa of human, plays an important role in development of peptic and duodenal ulcers as well as gastric cancer. The iceA, a member of the virulence genes of the bacterium, represents two distinct genotypes, iceA1 and iceA2. The iceA1 gene reveals strong homology to a restriction endonuclease (nlaIIIR) of Neisseria lactamica. This is relevant clinically because the strains harboring iceA1 genotype are associated with peptic ulcer disease (PUD) and increases acute neutrophilic infiltration. The purpose of this study was to produce the theoretical three-dimensional structure of IceA1 and IceA2 in order to perform a comparative structural analysis of two forms of the protein. The models were constructed using comparative and fold recognition modeling methods