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Poster Session Abstracts

The abstracts are presented in alphabetical order by the presenter's last name.

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Molecular Studies on Defense Genes Expressed in *Gossypium barbadense* Genotypes Infected with Cotton

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Seven different *Gossypium barbadense* genotypes cultivated in Lower Egypt were symptomology infected with cotton leaf curl virus disease. ELISA analysis was used to detect the presence of the virus in the collected plant tissues. Differential display-PCR was approached by using four different primers related to defense genes on the extracted mRNA, the results showed up-regulation of some genes in the infected genotypes while they were not observed in the healthy plants. The up-regulated bands were sequenced and the sequence analysis showed that they were chitinases and β-1,3-glucanase genes. For studying the gene expression in both the infected and healthy plants, real time PCR was performed. It was observed that the gene expression of chitinases gene in the infected samples was higher than the healthy as it ranged 1.54–3.4 while in the healthy it ranged 0.78–2.96 as well as β-1,3-glucanases gene was highly expressed in the infected samples as it ranged 0.38–2.98 while in the healthy samples it ranged 0.19–1.8. In conclusion: the gene expression was high in *Gossypium barbadense*-infected genotypes and decreased in the healthy, that indicates which infection with cotton leaf curl virus disease induces the expression of some defense genes to resist the viral replication and distribution in the host plants.

Biomarkers of Freshwater Algae *Lemna minor* as a Model of Urban Pollution with Pesticides and Heavy Metals

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Biochemical changes in algal tissues of *Lemna minor* were considered to be biomarkers for pesticides and heavy metals pollution in an urban district (Kafr El-Zayat, Egypt). Multiresidue of pesticides and potential toxic metals were examined in linear regression analysis with some biochemical components in algal suspension. Four contaminated sites were chosen for the biomonitoring program, while another site (rural zone) (S5) was considered a reference to compare the risk factors. The activity of Glutamic Oxaloacetic Transaminase (GOT) reached the lowest values in the winter season to account for 3.22, 6.75, 5.43, and 2.35 U. L-1 in Potato International Center (PIC) (S1), El-Nasaria (S2), Kafr Hashaad (S3), and Bonufer (S4), respectively, compared with S5 which did not exceed 4.80 U. L-1. On the other hand, during the same season, the activity of Glutamic Pyruvic Transaminase (GPT) showed a decrease only in S3 and S4, while it recorded the highest value (10.00 U. L-1) in S2. Carbohydrates and total protein levels significantly decreased in all sites compared with S5. The algal pigments reached the lowest values in S1 to account for 4.76, 1.83, 1.97 mg. L-1 for Chlorophyll a, b, and Carotenoids, respectively. Therefore, this study showed the importance of freshwater algae in biomonitoring programs especially in urban regions.

Using Genetic Transformation to Decrease Lignin Content in Maize

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Lignin is an aromatic biopolymer, an integral cell wall constituent in all vascular plants including the herbaceous varieties. Genetic Engineering using an antisense system offers a way to modulate enzymes in the lignin pathway which leads to a reduction in lignin. The plasmid pZMAS-C4H3 10.907bp has C4H3 antisense to down regulate O-methyltransferase and reduce lignin level in maize and bar gene as a selectable marker that confers glufosinate herbicide resistance under 35s promoter. Thirty-five transgenic lines expressed bar gene and C4H3 antisense. The results of this study pointed to the possible use of genetic transformation approach to minimize lignin content and consequently enhance bioethanol production from stover.

SNP VS Haplotype Associations for Mastitis Resistance in Dairy Cattle

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Mastitis is the most deleterious endemic disease facing dairy producers around the world. Therefore, mastitis resistance is an important breeding objective. To improve low heritable traits, such as mastitis resistance, genomic selection was suggested. As a first step in genomic selection, reliable knowledge of genetic markers in breeding populations is desirable. Although, methods based on SNP markers may lead to significant findings, analyses based on haplotypes comprising multiple SNPs on the same chromosome may provide additional power for mapping candidate loci and also provide insight on factors affecting the dependency among genetic markers. In this study, we evaluated the use of both SNP and haplotype markers through Genome-Wide Association Studies (GWAS) to identify the associated genomic loci. We used 2,360 German Holstein bulls for which daughter yield deviations for SCS were available. In addition, genetic information of 43,062 informative SNPs and 11,374 inferred haplotype blocks were used. After correction for false positive and negative associations, 16 SNPs and 12 haplotypes were identified (P≤1.16x10-06) and distributed on chromosomes 5, 6, 13, 18, 19, and X. The length of identified regions was between 0.05 Mb and 5.62 Mb. Loci on chromosomes 5, 6, 18, and 19 coincided with known QTL affecting SCS, while novel loci were found on chromosomes 13 and X. In all identified regions, except chromosome X, significant SNPs were contained in significant haplotypes. The combined effect of all significant haplotype blocks accounted for 5.3% of the total genetic variance, while all SNPs in those haplotypes explained only 4.0%. The results indicate that the application of GWAS using SNP- and haplotype-based approaches may maximize the potential for finding biologically important loci. Haplotypes captured more genetic variation than SNPs located in the haplotype blocks. The narrow regions identified in our study will facilitate the search for causal genetic variants affecting gene functions.

Construction of a Genetic Linkage Map for Durum Wheat

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The objective of this study is to dissect Quantitative Trait Loci (QTL) controlling grain yield, yield components, and drought tolerance in durum wheat. A molecular genetic linkage map for F2 durum mapping population derived from an intraspecific cross between Baniswif-1 x Sohag-2 was constructed using 114 DNA markers (9 SSRs, 14 SCoTs, 90 AFLPs, and 1 RAPDs) distributed over the 14 linkage groups and spanning 2040.9 cM of the durum wheat genome. The size of linkage groups varied greatly from 6.8 cM for LG11 to 317.5 cM for LG4 with an average length of 145.8 cM. Based on the used anchor SSR markers, only eight linkage groups were assigned to chromosomes, where LG1, LG3, LG5, LG6, LG7, LG9, LG13, and LG14 were assigned to chromosomes 1B, 3B, 5B, 6A, 6B, 7A, 3A, and 2B, respectively. This work represents the first genetic linkage map for durum wheat population derived from an intraspecific cross between Baniswif-1 and Sohag-2 showing chromosomal regions associated with 11 morpho-physiological traits related to grain yield, yield components, and drought tolerance.

Targeting Wnt/β-Catenin Pathway as a Potential Treatment in Breast Cancer Invasion

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In breast cancer, a complex molecular interplay involving multiple signaling pathways–including Wnt/β-catenin signaling cascade–has been attributed to the development of this disease. In most cases, Wnt/β-catenin signaling is thought to drive tumorigenesis through the stabilization of cytosolic β-catenin, which goes into the nucleus, binds to a family of transcription factors, and initiates a new gene expression program. Moreover, Wnt/β-catenin pathway has been found to play a role in the activation of Epithelial-Mesenchymal Transition (EMT), in which epithelial cells lose cell–cell contacts and acquire mesenchymal characteristics, facilitating their detachment from the primary tumor, invasion through the basement membrane, and entry into the circulation.

Aim: In this study, we investigated the effects of Indomethacin, a non-steroidal anti-inflammatory agent, which has been known for its chemo-preventive effects in different types of cancers, on Wnt/β-catenin signaling, apoptosis induction and EMT in breast cancer in vitro and in vivo models.

Methods: In vitro, we examined the effect of Indomethacin on human breast cancer cells T-47D and MCF-7 proliferation and apoptosis, and the expression levels of β-catenin and anti-apoptotic Bcl-2 in these cell lines, using western blot. In vivo, we examined the effect of Indomethacin on albino rats' mammary tumors development, and the expression levels of β-catenin, and epithelial marker E-cadherin, using immunohistochemical analysis.

Results: Indomethacin inhibited cells viability and induced apoptosis in human breast cancer T-47D and MCF-7 cells, with reduction in β-catenin, and Bcl-2 expression patterns in both cell lines. Indomethacin also reduced the albino rats' mammary tumors sizes in the treated group to 40% compared to the untreated group, and inhibited the expression of β-catenin and enhanced the expression patterns of E-cadherin.

Conclusion: Indomethacin has strong inhibitory effect on Wnt/β-catenin signaling and inhibited breast cancer progression in both breast cancer in vivo and in vitro models.

Genetic and Phenotypic Characterization of *Moraxella catarrhalis* Strains Isolated from Egyptian Otitis Media Children

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Moraxella catarrhalis, an opportunistic pathogen of both the upper and lower respiratory tract, is the third most common bacterial cause of childhood otitis media. The aim of this study is to characterize the *M. catarrhalis* surface antigens and the antibody response that they elicit in Egyptian children that are diagnosed with otitis media. To this end, 135 nasal swabs, saliva, and blood samples were collected from children suffering from otitis media and 50 from non-otitis media ones. Biochemical and genetic identification schemes indicated that 24 (18%) of the otitis media samples have *M. catarrhalis* strains versus 21 (40%) from the non-otitis media cases. Phenotypic characterization of these *M. catarrhalis* isolates collected from both groups of children was performed using an assay of biofilm formation, autoagglutination, and serum resistance. PCR was also performed to look for some of the genes that may contribute to virulence of the *M. catarrhalis* such as uspA1, uspA2, uspA2H, hag, copB, ompCD. In all the experiments the percentages of the strains giving positive phenotype are higher among *M. catarrhalis* strains isolated from otitis media children. For instance, 75%, 62%, and 88% of the otitis media were positive in biofilm formation, auto agglutination, and serum resistance, respectively. On the other hand, only 70%, 46.5%, and 77% of the non-otitis media *M. catarrhalis* strains were positive in the same assays. Ongoing work focuses on assessing the humoral immune response elicited in both groups against the major *M. catarrhalis* surface antigens to determine if it plays a role in protection against the disease. After comparing the results it may be determined whether *M. catarrhalis* induced otitis media correlates with virulence of *M. catarrhalis* isolates in the nasal tract or an unfavorable M Cat-specific host antibody response.

Investigation of the Potential Utility of Metformin in Combination with Either Chemotherapy or Hormonal Therapy in the Treatment of Breast Cancer

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Introduction: Metformin, one of most widely prescribed oral hypoglycemic agents, has recently received increased attention because of its potential anti-tumorigenic effects in different cancer types, which are attributed to several mechanisms.

Purpose of the Work: to determine whether the addition of metformin to systemic therapy in non-diabetic breast cancer female patients has an effect on decreasing recurrence and metastasis via reduction of circulating glucose, insulin levels, insulin resistance, and alteration of Insulin Growth Factor (IGF) signaling.

Patients and Methods: a total of 100 eligible women with diagnosed breast cancer from Damanhur Oncology Center in Egypt, aged between 40 years old to 60 years old with performance status 0-1. Eastern Cooperative Oncology Group (ECOG) was divided into two main groups (control group and metformin group) each group was divided into four sub-groups according to breast cancer stage. Women in the metformin group were administered systemic treatment in addition to metformin 850 mg twice daily. Fasting blood samples were collected from patients with breast cancer at the baseline, after chemotherapy, after 6 months and one year of hormonal therapy. Fasting serum glucose, insulin, Insulin-like Growth Factor-1 (IGF-1), Insulin-like Growth Factor Binding Protein 3 (IGFBP3) and Caner Antigen 15.3 (CA15.3) were evaluated. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and IGF-1 to IGFBP3 ratio (bioactive IGF-1) were also determined.

Results: Administration of metformin shows a significant reduction of insulin, glucose, HOMA-IR and IGF-1 to IGFBP3 ratio. It also shows a significant increase in IGFBP3, while it has a non-significant effect on IGF1, and CA15.3 in stage 1 and 2 compared to the control group. A decrease in the number of breast cancer metastasis cases in stage 3 versus control group is noticeable.

Conclusion: Metformin may have a beneficial effect in breast cancer management and metastasis.

A Multiplex PCR for Rapid Typing of Group A *Streptococci* According to hage-encoded Streptodornases

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Group A *Streptococci* (GAS) are strictly human pathogens that are behind the death of 650,000 humans every year because of invasive infections. Recently, the emergence of hypervirulent, invasive GAS strains has been correlated with the carriage of variants of the streptodornase (sda) gene. Streptodornases are secreted toxins that protect the bacteria from being trapped in Neutrophil Extracellular Traps (NETs) by degrading the nucleic acids that form these traps. So far, five major classes of streptodornases have been discovered, four of which are phage-encoded: Spd2, Spd3, Spd4, and Sda. Whereas variants of the chromosomally encoded streptodornase, Spd, are present in all streptococcal genomes, the distribution of phage-encoded streptodornases follow different patterns that do not correlate with the emm type of an isolate. To rapidly investigate the distribution of phage-encoded streptodornases among clinical isolates, we initiated a study to develop a PCR-based approach for typing clinical GAS isolates. Isolates were cultured on blood agar and trypticase soy broth, tested for nuclease activity by subculture on DNA-agar plates containing methylene blue, then by an agarose gel electrophoresis-based method to test the extent of DNA degradation. Genomic DNA from the clinical isolates was extracted. PCR primer pairs were designed and tested for each of the five streptorodnase genes. After all primer pairs had been verified and confirmed to be specific and sensitive, a multiplex PCR was optimized to test all five genes in one reaction. Using samples with known sda distribution patterns, we confirmed the ability of the newly developed reaction to resolve different patterns without cross-reactivity or non-specific products. Our multiplex PCR method proved highly specific while saving time and money. In conclusion, we screened clinical GAS isolates representing different M serotypes for their nuclease activity by two methods, and we successfully developed a multiplex PCR for streptodornase typing of clinical GAS isolates.

Novel Antimicrobial Nanofiber-Reinforced Chitosan Membrane for Enhanced Healing of Infected Wounds

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Wound infection by *Staphylococcus aureus (S. aureus)* is a frequent post-surgery complication. Our aim is to assess the potentials of a novel composite antimicrobial nanofiber-reinforced chitosan membrane in the healing of *S. aureus*-infected incision wounds in rats. The membrane consists of a 2% chitosan film mechanically reinforced with Polycaprolactone (PCL) Nanofibers (NFs) loaded with Doxycycline hydrochloride (Doxy). NFs (≈180 nm), which were fabricated by emulsion electrospinning of a biodegradable PCL/polyethylene glycol (PEG) copolymer. Doxy release was maintained for two weeks. Healing was monitored morphologically, microbiologically, histologically, and by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Data obtained after two weeks indicated that the Doxy-composite membrane enhanced wound healing in terms of speed and quality compared to non-medicated composite membranes. Microbiological monitoring revealed maintained wound cleanliness over the study period due to controlled Doxy release. Histological examination demonstrated enhanced collagen deposition and maturation with complete re-epithelialization. At the molecular level, wounds treated with non-medicated or Doxy-composite membrane showed a significant decrease in mRNA of matrix metalloproteinase-1 and an increase in Transforming Growth Factor-Beta1 (TGF-ß1), Vascular Endothelial Growth Factor (VEGF) and Cyclooxygenase-2 (COX-2). A microRNA induced by hypoxia, MiR-210, was significantly reduced in wounds treated with non-medicated and Doxy-composites. This was associated with a significant increase in transcription factor E2F3 involved in cell cycle regulation. In addition, slow degradation of chitosan, probably allowing spacing between nanofibers and promoting cell infiltration within the NFs matrix, provided a large surface area for cellular interactions. The novel Doxy-composite membrane combines the wound healing accelerating effects of Doxy, NFs, and chitosan membrane all of which orchestrated healing promotion. The composite offers promise as a drug delivery scaffold for biomedical applications involving both cell regeneration and infection control, such as the healing of infected wounds.

Polymorphisms in Genes Encoding Drugs Metabolizing Enzymes among Egyptian Acute Lymphoblastic Leukemia Patients

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Acute Lymphoblastic Leukemia (ALL) is the most common malignancy of childhood. The backbones of the chemotherapy of ALL treatment, methotrexate (MTX) and 6-mercaptopurine (6-MP), have known, direct, adverse effects on hepatic functions. Several enzymes are involved in the metabolism of both drugs. At the same time, the genes encoding these enzymes are associated with genetic polymorphisms, which may contribute to the risk of developing cancers. Up to this point, very few studies have been concerned with the genotyping of Egyptian patients in the polymorphic genes that are involved in the metabolism of chemotherapeutic agents. To this end, 29 ALL patients were studied to determine the genetic polymorphisms in three classes of enzymes: Glutathione S-Transferase (GST), Methylenetetrahydrofolate Reductase (MTHFR), and Thiopurine S-Methyltransferase (TPMT). Using PCR and Restricted Fragment Length Polymorphism (RFLP), we found that 82.8% have either homozygous or heterozygous GSTM1/GSTT1 mutated allele and none have the wild-type GSTP1 allele. For the MTHFR, 55.2% have either homozygous or heterozygous C677T mutated allele. Finally, in the case of TPMT, neither the G238C nor the G460A mutated allele was found in any of the cases. Ongoing work is focused on studying the possible role these genetic polymorphisms might have on the therapeutic outcome of these patients. Efficient screening of the Egyptian ALL patients population in Egypt is crucial for determining the proper dosing and treatment regimens of both MTX and 6-MP.

Overexpression of AcrAB Efflux Pump and the Role of Mefloquine as an EPI in MDR *E. coli* Isolates

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Multi-drug resistant *Escherichia coli* (MDR *E. coli*) are widely distributed in hospitals which are increasingly isolated from community, representing a major health problem. The AcrAB pump constitutes a major drug efflux system. Inhibition of efflux pumps appears to be an attractive approach to combat drug resistance problem. Mefloquine was confirmed to be a strong bacterial RND Efflux-Pump Inhibitor (EPI). This study aims to investigate the expression levels of multidrug efflux genes acrAB, as well as to study the effect of Mefloquine as an efflux pump inhibitor. AcrA and acrB gene expression was measured in clinical isolates using real-time PCR. The effect of EPI, mefloquine hydrochloride, on Minimum Inhibitory Concentration (MIC) of selected antibiotics against the tested isolates was determined using checkerboard broth microdilution technique. Overexpression of both genes was detected in all isolates. The differences between expression levels of both genes in MDR strains and ATCC strain were statistically significant ($p < 0.001$). There was a strong correlation between acrA and acrB genes expression levels. ($r = 0.593$, $p < 0.001$). The isolates showed decrease in MIC of tested antibiotics in presence of EPI. Susceptibility to Levofloxacin was recovered in 95% of the tested isolates, while in 62.5% to Ceftriaxone, and in 5% each to Ciprofloxacin and Gentamicin in presence of Mefloquine. In conclusion, inhibition of acrAB efflux pump in MDR *E. coli* by Mefloquine as EPI needs to be considered in the design of future antibiotics. However, further studies exploring this novel strategy of interfering with efflux pump expression and function are warranted.

HPLC-DAD and HPTLC Methods for Simultaneous Determination of Carvedilol and Hydrochlorothiazide

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Two chromatographic methods were developed for the simultaneous determination of the antihypertensive drugs Carvedilol (CRV) and Hydrochlorothiazide (HCT) in their combined tablet dosage form.

Method I: The reversed phase HPLC method was performed using Zorbax SB-C8 column (4.6×250 mm, 5 µm particle size) with gradient elution of the mobile phase composed of 0.025 M phosphoric acid and acetonitrile at a flow rate of 1 mL/min. The Diode Array Detection (DAD) was utilized as a tool for peak identity and purity confirmation. The multiple wavelength detector was set at 242 nm for measurement of CRV and 271 nm for HCT at retention times 4.9 and 6.7 min for HCT and CRV, respectively. The linearity ranges were 5–300 µg/mL and 5–200 µg/mL for CRV and HCT, respectively, with correlation coefficients > 0.9996. Both drugs were subjected to stress conditions of hydrolysis, oxidation, photolysis, and thermal degradation. The proposed HPLC-DAD method proved to be stability-indicating by resolution of the drugs form their forced degradation products.

Method II: The HPTLC analysis was carried out on an aluminum-backed sheet of silica gel using chloroform and methanol in the ratio of 8:2 v/v as the mobile phase. Quantification was achieved with UV densitometry at 254 nm for both CRV and HCT where the linearity ranges were 0.05–1 µg/spot and 0.1–2 µg/spot for CRV and HCT, respectively, with correlation coefficients > 0.9997. The analytical performance of both methods was thoroughly validated according to International Conference on Harmonization (ICH) guidelines with respect to system suitability, linearity, ranges, precision, accuracy, specificity, robustness, detection, and quantification limits. The proposed HPLC and HPTLC methods were successfully applied to the determination of both drugs in laboratory-prepared mixtures and in their combined tablet dosage form. No chromatographic interference was observed from the tablets excipients.

Preventing the Next Superbug: The Egyptian Hospital Resistome Project and Database

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The emergence of AntiMicrobial Resistance (AMR) is a global health threat, particularly for microbes causing healthcare-associated infections, which have alarmingly become among the top leading causes of death. Interestingly, patterns of antibiotic resistance differ widely among countries. Therefore, knowledge of local patterns is vital to guide empirical and microbe-specific treatment. Although a substantial number of studies have been published on multiresistant pathogens—also known as superbugs—isolated from Egyptian patients, there is a sparsity of data on the spread of multi-resistant microbes and AMR genes in the hospital environment (such as beds, door knobs, patient trays). Here, we describe a pilot project that aims at a large-scale surveillance of the Egyptian hospital resistome (defined as the collection of AMR genes and the microbes that carry them in the hospital environment). The project is modeled after the international hospital microbiome project but focuses on the detection of multiresistant microbes and AMR genes using culture-based and culture independent techniques. So far, samples have been collected from different inanimate objects in Abo El-Rish Children's Hospital. Preliminary antimicrobial sensitivity screenings show striking differences between samples collected from the radiology, blood collection, and intensive care units. For example, whereas radiology unit-isolated microbes are resistant to vancomycin and cefotaxime, but susceptible to levofloxacin and kanamycin, those isolated from the blood collection unit were only resistant to vancomycin but highly sensitive to levofloxacin. Bacterial DNA was successfully isolated and is expected to get sequenced. The project aims to intelligently monitor the current AMR status to prevent the emergence of new resistance through preemptively directing the appropriate use of antibiotics according to the local resistance. This pilot project will be the nucleus for the construction of a database documenting the distribution of resistance genes in Egyptian hospitals, hence, the foundation for the Egyptian Hospital Microbiome Project.

Bioavailability of Heavy Metals in Fresh Water *Tilapia nilotica* (Oreachromis niloticus Linnaeus, 1758): Potential Risk to Fishermen and Consumers

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Continuous exposure to small doses of heavy metals can trigger numerous reactions in human beings. This study focuses on the accumulation progress of some heavy metals (Cr, Co, Cu, Ni, Zn, Pb and Cd) in different tissues (muscle, gills, heart, liver, brain, bone and skin) of *Tilapia nilotica* collected during the last four months, before their final collection for commercial marketing, in relation to their potential risk on consumers. This fish farm is fed with discharged water containing agricultural, industrial, sewage and domestic wastes. The length-weight relation and condition factor calculation of *Tilapia nilotica* samples showed a significant linear regression ($r2 = 0.920$) and an average condition factor of 4.1 g/cm3. This indicated that the health status for the studied fish samples was good. Metal Pollution Index (MPI) values in the different tissues reflected that the muscle was the only tissue that had the lowest content. Provisional Tolerable Weekly Intake (PTWI) values for the investigated heavy metals were lower than those reported for the permissible limits. The data were evaluated by using ANOVA statistical analysis. Human health risk effects of heavy metals in fish muscle, Estimated Dietary Intake (EDI), and Hazard Quotient (HQ) were determined. HQ levels indicated that Cr and Co were the only heavy metals among the determined ones that had more values than units. Also, their relative contributions in fish consumptions were Cr> Co> Pb> Ni> Cu> Cd> Zn. The highest average HQ value of chromium determined in this study referred to the possible adverse effects of Cr on human health. In general, heavy metals could pass to humans through the food chain and thus predispose the consumers to possible health hazards. Periodic monitoring of heavy metals in both the fishes and fish farm system to ensure continuous safety of people in the area is recommended.

Methylene Blue-Nanofibers for Photodynamic Therapy of Infected Wounds in Immunocompromized Rats

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Wound infections pose a significant health burden which frequently contributes to poor health-related quality of life and loss of productivity. Furthermore, the increasing resistance of wound infections to both systemic and topical antibiotics may contribute to delayed healing, morbidity, and mortality. In this study, we propose a novel therapeutic modality for wound healing which aims to combine the effective and likely microbial resistance-free Antimicrobial Photodynamic Chemotherapy (APDT) with the cell-regeneration promoting effects of nanofibrous wound dressing. Methylene Blue (MB), a water soluble photosensitizer with multiple biological effects, was encapsulated into Polyhydroxybutyrate/Polyethylene Glycol (PHB/PEG) blend Nanofibers (NFs) prepared by emulsion electrospinning after adjustment of formulation and process variables. Controlled release profile of MB matched wound infection control needs. Assessment of MB-NFs as wound dressing biomaterial for *Staphylococcus aureus* (*S. aureus*)-infected excision wounds was performed in immunocompromized rats. Morphometrical, microbiological, and histopathological data indicated enhanced wound repair. Multiple irradiation of MB-NFs treated wounds with red LED at optimum wavelength and adjusted treatment conditions resulted in a prompt eradication of wound infection. Accelerated wound closure without scar formation was observed at the macroscopic level. RT-PCR analysis of wound bed samples revealed upregulation of the growth factors PDGF, VEGF, as well as Cox-2 m-RNA expression; all contributing to the improved quality of the healed tissues as observed in the histopatholological analysis. The latter showed enhanced granular tissue formation, reepithelialization, modulated matrix formation, and improved angiogenesis of the wound bed. Findings provided a proof of concept for the combined potentials of PACT and MB-NFs as a biomaterial for enhanced wound repair rate and quality, as well as infection control, in the challenging immunocompromized animal model used.

Differential Gene Expression of Tumor Cell in Response to Different Thalidomide Dithiocarbamate Analogs

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Thalidomide has been a target of active investigations in both malignant and inflammatory conditions. The aim of this work is to investigate some novel thalidomide analogs for their cytotoxicity, anti-angiogenic, anti-inflammatory, antioxidant, treratogenic gene (FGF-2) effect, histone deacetylase (HDAC) activity, and nuclear factor kappa B (NF-κB) level using two different cell lines (Hep-G2 and MCF-7 cells) and macrophage cells. Analogs 1, 3, and 5 showed elevation in their cytotoxic activity better than thalidomide. Conversely, Analog 3 has the lowest cytotoxic activity in MCF-7 cells, while all the other Analogs have comparable cytotoxic activity. Analogs 4 and 2 showed reduction in pro-angiogenic and elevation of anti-angiogeneic factors in both cell lines. Furthermore, Analog 1 is a potent Analog in MCF-7 cells as an anti-angiogenic agent. Analogs 5 and 1 showed a potent, anti-inflammatory effect. Hep-G2 cells exposed to different thalidomide compounds (thalidomide analogs 1, 2, and 4) showed an increase in NF-κB level, while Analogs 3 and 5 decreased NF-κB level in relation to thalidomide. In MCF-7 cells, comparing all analogs revealed that they produced comparable amounts of NF-κB with the lowest production in response to Analog 2. Analog 2 has potent inhibition to HDAC. However, all the other Analogs have less ability to reduce HDAC activity compared to thalidomide. Analog 4 has a teratogenic effect on both cell lines while Analog 5 has a teratogenic effect on Hep-G2, and Analogs 1, 2, and 3 have a teratogenic effect on MCF-7 cell. All Analogs showed pro-oxidant activity against ORACOH• while, Analog 1 and Analog 3 revealed pro-oxidant activity against ORACROO•. Analog 4 showed a potent scavenging capacity against ORACROO•. Together, all of the data revealed that Analog 4 and Analog 2 have an obvious anti-cancer effect on both cell lines (Hep-G2 and MCF-7). Analog 5 and Analog 3 exert their main effect on Hep-G2, while Analog 1 has a better effect on MCF-7 cells.

Phenotypic and Genotypic Method for Detection of MexEF Opr-N Efflux Pump in Pseudomonas aeruginosa

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P. aeruginosa is a leading pathogen that causes Health-care-Associated Infections (HAIs). It has acquired multiple mechanisms of resistance against all available anti-pseudomonal agents. The efflux-mediated resistance mechanisms confer a moderate level of resistance and are often multi-drug resistant. Its clinical impact could be important, because it may make antibiotics inefficient in infected sites, and it confers cross-resistance to unrelated antibiotic classes. In this study, a combined phenotypic and genotypic approach was used for the diagnosis of resistance mediated by MexEF-OprN efflux pump among clinical and environmental isolates of *P. aeruginosa*. Phenotypic detection used MIC measurements of Levofloxacin (LVX) with and without Phe-Arg-β-naphthylamide. Genotypic detection was made by reverse transcription polymerase chain reaction to detect the expression of the MexEF-OprN gene. All the 50 LVX resistant *P. aeruginosa* isolates had cross resistance to the Floroquinolones (FQ) group of antibiotics included in the study (norfoxacin, ciprofloxacin and ofloxacin). Among the antibiotics tested, Imipenem (IPM) was the most active against LVX resistant *P. aeruginosa* (42%) followed by Cefepime (FEP) (20%), and the other antibiotics' activity ranged from 0% to less than 20%. Twenty-one isolates showed complete convergence (20 P+ G+ and 1 P- G-). Partial conversion was observed in three strains (P- G+) and divergence was observed in three strains (P+ G-). Inhibition of efflux systems with broad-spectrum inhibitors would seem to be a prudent approach to combat and/or prevent FQs resistance or even multi-drug resistance.

Keywords: *P. aeruginosa*, MexEF-OprN efflux pump, fluoroquinolones resistance.

Metabolome Classification of *Chrysanthemum pacificum* Nakai Organs via UPLC-QTOF-PDA-MS and Chemometrics

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The genus *Chrysanthemum L.* (*Asteraceae*) comprises about 40 species, most of which are recognized as potential hepatoprotective, anticancer, antihypertensive, and antioxidant agents. Among them, *C. pacificum Nakai*, native to Japan, received scant characterization in terms of either its phytochemical composition and/or its biological effects. In this study, we have utilized high-throughput metabolomics technologies, using UPLC-QTOF-PDA-MS combined with intensive computational and statistical analysis, to conduct comprehensive metabolite profiling of the aerial parts, roots, and flowers of *C. pacificum Nakai* plants grown in Egypt. Identified metabolites belonged to various classes including hydroxycinnamic acid conjugates and flavonoids. Principal component analysis of derived biochemical profiles was also used for organism classification. Flowers were characterized by enrichment in hydroxycinnamic acid conjugates, whereas variation in the accumulation pattern of metabolites from flavonoids was observed among organisms, particularly in the case of flavonols (myricetin, quercetin and isorhamnetin), flavones (luteolin) and their methyl ethers and glycosidic conjugates. These findings provide the most complete map for polyphenols composition in *C. pacificum Nakai*. By describing the metabolites profile in *C. pacificum* and identifying potential biomarkers for its quality assurance, this study provides the basis for future investigations of its different organisms for potential biological and or nutritional use.

STAT5a Signaling, Novel Strategy for HCC Treatment

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Hepatocellular Carcinoma (HCC) ranks as the fifth most common solid tumor worldwide and the third cause of cancer-related death. Signal Transducers and Activators of Transcription (STAT) play different cellular functions, such as being signal transducers in the cytoplasm and transcription activators in the nucleus. Each STAT gene shows different functions; we focused only on the roles of the STAT5A and STAT3 genes in the HCC progression. The aim of our study is to find a new treatment for HCC progression and investigate the role of the STAT family, including STAT5a and STAT3 expressions in the HCC. Herein, we examined the effect of a newly formulated (SCP1) compound [Silver cyanide trimethyltin1,2-Bis(4-pyridyl) ethane] Supramolecular Coordination Polymer (SCP) on HCC progression and downstream signal profile of STAT3, STAT5a *in vitro*.

Methodology: We examined the effect of SCP1 compound on both STAT5a, STAT3 signaling in two HCC cell lines, HepG-2 and Hep3-B respectively, and then investigated the expression of both cell differentiation and proliferation markers in HCC. We also evaluated the effect of the SCP1 compound on various mediators of cellular proliferation, cell survival, and apoptosis signaling, using STAT5a gene adenovirus (wt) transfection to confirm the role of STAT5a gene in HCC, which could be a potential application of STAT5a as a therapeutic target to HCC treatment.

Results: Our study provides novel evidence that combined treatment of STAT5a and SCP1 compounds plays a pivotal role in induced apoptosis in treated HCC compared to control and displayed morphological changes with pre-apoptotic characteristics, including cell proliferation inhibition, β-catenin, Bcl-2 inhibition and DNA fragmentation *in vitro*.

Conclusion: Active wt-STAT5a transfectants of HCC cells induced apoptosis and inhibited β-catenin and Bcl-2 expression, providing novel evidence that wt-STAT5a and SCP1 compound synchronized to enhance cancer cells for differentiation and apoptotic induction in treated HCC cell lines.

High Performance Liquid Chromatographic (HPLC) Determination of the Ternary Mixture of Caffeine, Dipyrone and Drotaverine Hydrochloride (HCl) in Tablets Dosage Form

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This work describes a simple, rapid, and reliable HPLC method for the simultaneous determination of caffeine (CAF), dipyrone (DIP) and drotaverine hydrochloride (DRV). Chromatographic separation was achieved using a reversed phase Waters Symmetry C18 (3.9×150 mm, 5 µm particle size) column with gradient elution of the mobile phase composed of 0.05 Morthophosphoric acid and acetonitrile. The gradient elution started with 15% (by volume) acetonitrile ramped up linearly to 60% in 3 minutes then kept at this percentage till the end of the run. The flow rate was 1 mL/min. Quantification was based on measuring peak areas at 210 nm. The analytes were resolved with retention times 1.47, 2.39 and 7.17 min for DIP, CAF and DRV, respectively. Analytical performance of the proposed procedure was validated with respect to system suitability, linearity, ranges, precision, accuracy, robustness, detection and quantification limits. The linearity ranges were 10–200, 5–100 and 5–100 µg/mL for DIP, CAF, and DRV, respectively. The validated HPLC method was applied to the simultaneous determination of the three drugs in several laboratory-prepared mixtures of different ratios. Finally, the laboratory-made tablets containing the three drugs were assayed using the developed procedure where no interfering peaks were encountered from the tablet additives.

Isolation and Production of Antifungal Compound(s) against Fungi Associated with Diabetic Foot Ulcer

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Diabetes mellitus is a major and growing health problem in the world. Diabetes without proper management can cause many complications. Foot ulcers are one of the main serious complications of the disease. Diabetic foot ulcers are a leading cause of hospitalization and amputation. Diabetic foot ulcer is a polyetiological disease. In some diabetic patients, *Candida* and dermatophyte infection may play a significant role in its pathogenesis. Twenty-one microbial samples were collected from diabetic foot ulcer patients on Sabouraud dextrose agar. Several identification methods (morphological, chromogenic medium, and molecular methods) were used to identify some fungal isolates. Results indicated that *Candida* species were the most frequent fungal isolates. *C. albicans* was the most dominant strain followed by *C. tropicalis* and *C. dubliniensis*. The nucleotide sequence of one yeast sample was deposited in the GenBank sequence database and given the accession number KC936147.1. A second yeast isolate was found to have high similarity with *C. tropicalis*. Again, the nucleotide sequence was deposited in the GenBank sequence database and given the accession number KC899707.1. Several bacterial isolates were screened for their antifungal activity against *C. albicanis*. The most potent strain was *B. subtilis* GenBank accession No. EF583053. The production of antifungal compound was monitored during the growth of *B. subtilis* reference strain. The production of the *B. subtilis antifungal* was optimized through several steps (OVAT, Plackett–Burman, and response surface methodology). In an attempt to characterize the antifungal compound produced by *B. subtilis,* the heat stability was tested and treated with proteolytic enzyme (alkaline protease). This study reflects on the one hand, the importance of microbiological tests for the treatment of diabetic foot ulcer, and on the other hand the ability of some microorganisms to produce natural antifungal agents effective against fungi-associated with diabetic foot ulcer.

Thymoquinone (TQ) and/or Tamoxifen Synergistically Inhibit Human Breast Cancer Cell Lines Proliferation

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Thymoquinone (TQ) is the bioactive constituent of the volatile oil of the black seed (*Nigella sativa*) and has been shown to exert anti-inflammatory, anti-oxidant, and anti-neoplastic effects both *in vitro* and *in vivo* against human pancreatic adenocarcinoma, uterine sarcoma and leukemic cell lines, while it is minimally toxic to normal cells. The aim of this study is to investigate the role of TQ alone and/or in combination with Tamoxifen (TAM) on human breast cancer cell lines MCF-7 and T-47D respectively. Herein, we investigated the effect of TQ alone or in combined treatment with TAM on MCF-7 and T-47D breast cancer cells by conducting MTT assay, DNA fragmentation Assay, Western blot analysis, Immunocytochemistry, ELISA assay for active Caspase-3, and microscopic examination for morphological changes. Our data revealed that TQ alone shows inhibition of growth of MCF-7 and T-47D cell lines by down regulation of Signal Transducer and Activator of Transcription 3 (STAT3) and its target proteins in a dose dependent manner. STAT3 is found in 30%–60% of primary breast cancers and is associated with enhanced proliferation, resistance to apoptosis, cell movement, invasion and metastasis. TQ treatment alone and/or in combination with TAM down regulated B-catenin signaling, which was consistent with cancer cell growth inhibition and apoptosis induction through Caspase-3 activation and BCL-2 down regulation. Furthermore, we found that the co-treatment of both TQ and TAM results in a synergetic effect that leads to a more effective growth inhibition of tested cell lines and a high percentage of apoptosis induction. For your knowledge, we report for the first time that TQ alone and/or TAM showed synergetic effect to inhibit breast cancer cell line proliferation through down-regulation of STAT3 and B-catenin to control breast cancer progression.

The Combined Effect of Metformin and L-cysteine in STZ-Induced Type 2 Diabetes in Rats

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Increasing evidence has established causative links between obesity, chronic inflammation and insulin resistance; the core pathophysiological feature in type 2 diabetes mellitus. This study was designed to examine whether the combination of L-cysteine and metformin would provide additional benefits in reducing oxidative stress, inflammation and insulin resistance in streptozotocin-induced type 2 diabetes in rats. Male Wistar rats were fed a High-Fat Diet (HFD) for 8 weeks to induce insulin resistance, after which they were rendered diabetic with low-dose streptozotocin. Diabetic rats were treated with metformin (300 mg/kg/day), L-cysteine (300 mg/kg/day), and their combination along with HFD for another two weeks. Control rats were fed normal rat chow throughout the experiment. At the end of treatment, fasting blood glucose, fasting serum insulin, Homeostasis Model Assessment–Insulin Resistance Index (HOMA-IR), and serum Free Fatty Acids (FFAs) were measured. Serum levels of the inflammatory markers; Monocyte Chemoattractant Protein-1 (MCP-1), C-Reactive Protein (CRP) and nitrite/nitrate were also determined. The liver was isolated and used for determination of Malondialdehyde (MDA), reduced Glutathione (GSH), caspase-3, and cytochrome c levels. The hypoglycemic effect of the combination therapy exceeded that of metformin and L-cysteine monotherapies with more improvement in insulin resistance. All treated groups exhibited significant reductions in serum FFAs, oxidative stress and inflammatory parameters, and caspase-3 and cytochrome c levels compared to untreated diabetic rats with the highest improvement observed in the combination group. In conclusion, the present results clearly suggest that L-cysteine can be strongly considered as an adjunct to metformin in the management of type 2 diabetes.

Targeting Egg Production in the Egyptian Cotton Leafworm: A New Approach to Insect Control

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Egg laying arthropods deposit large amounts of nutrients into their eggs to ensure the completion of embryonic development. Such nutrients include proteins, lipids, carbohydrates, minerals, and other compounds. Vitellogenin (Vg) is the precursor of Vitellin (Vn) which is the major yolk protein in eggs. In insects, Vg is usually synthesized in the fat body, secreted into the hemolymph, and taken up by the ovary to be incorporated into growing oocytes via receptor (Vitellogenin Receptor [VgR]) mediated endocytosis. Both Vg and VgR genes are crucial for the egg production. In this report, we describe the isolation and characterization of the Vg and VgR genes from the Egyptian cotton leafworm, *Spodoptera littoralis*, which is a major pest in Egypt. Developmental studies, tissue specificity, and the use of both genes in pest control are also discussed.

Mycoremediation of Plastic Wastes is Our Challenge for the Next Decade

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Fungi are known to degrade, or deteriorate, a wide variety of materials and compounds. These processes are known as mycodegradation and mycodeterioration. Plastic is one of the most common synthetic polymers used in various applications such as coatings, fibers, paints, and packaging. Although, plastic has many advantages (such as it being lightweight, low cost, and highly durable), it causes various threats to the environment due to its non-degradable nature and lack of safe disposal sites (landfills) in Egypt. Therefore, new techniques for the remediation of plastic wastes should be used based on degradation. This study aims to investigate the capabilities of local fungal isolates on plastic degradation in vitro. Fourteen genera and twenty species were recovered from twenty-five soil samples were collected from different plastic waste disposal sites in Egypt. The plastic degradable fungi were isolated and identified by morphological and molecular biology means. Out of the fourteen genera recovered, genus Aspergillus was the most frequent. A. versicolor, A. sydowii, A. terreus, A. ochraceus, A. candidus, A. niger, and A. flavus were screened for the production of extracellular enzymes as one of the most important mechanism for plastic degradation. Modeling experiment has been designed for biodegradation of synthetic plastic sheet by the isolated taxa. Plastic degradation was estimated by different techniques such as weight loss, Scanning Electron Microscopy (SEM), and Fourier Transform Infrared spectroscopy (FTIR) spectroscopy. Different trails to immobilize potential fungal taxa and the effects of pH and co-substrates were investigated at different contact times.

Keywords: *Aspergillus*, Egypt, Enzymes, Immobilization, Mycodegeradation, Plastic waste management.

Chemometric Determination of Ephedrine-HCl Impurity in Pseudoephedrine-HCl Raw Material Using FT-IR

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Ephedrine-HCl and pseudoephedrine-HCl are both sympathomimetic agents that possess vasopressor effect. Ephedrine-HCl is mainly used as a bronchodilator in asthma preparations while pseudoephedrine-HCl is used as a nasal decongestant. The British pharmacopoeia stated a limit of 1% of ephedrine-HCl impurity in pseudoephedrine-HCl pharmaceutical raw material [1]. Ephedrine-HCl and pseudoephedrine-HCl are diastereomers that possess the same UV spectral features. Hence, their simultaneous spectrophotometric determination is practically impossible. Chemometric multivariate methods, Principal Component Regression (PCR), and Partial Least Squares (PLS) were applied to the simultaneous determination of both isomers using Fourier Transform Infrared Spectrometry (FT-IR). The training set was constructed using a full factorial calibration design at four levels. Both multivariate calibration models were developed using the correlation between the concentration and the absorbance data matrices in two spectral regions: the non-specific region (3260–2790 cm^{-1}) and the finger print region (1450–850 cm^{-1}). The two isomers possess very slight variations in the IR spectra at the selected regions that can be utilized by the chemometric models to generate the calibrations. The methods were validated by analyzing an independent validation set. The methods were found to be accurate and precise as indicated by the mean percentage recovery (100.19%–100.67%) and percentage relative standard deviation (0.75%–1.03%) respectively. The methods were successfully applied to the determination of trace ephedrine-HCl impurity in pseudoephedrine-HCl bulk raw material within the British pharmacopoeial limit without any prior separation step. The results were statistically compared to those obtained using the BP HPLC reference method.

Pharmacogenomic Approach to Study Resistance to FAC Therapy among Egyptian Breast Cancer Patients

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Despite the expanding repertoire of new anti-cancer drugs, therapy resistance remains an almost inevitable outcome and major challenge to oncologists in the treatment of breast cancer. In searching for an answer to the multidrug resistance phenotype, many studies have focused on the MDR1 gene which encodes P-glycoprotein. P-glycoprotein over-expression in tumors confers resistance to a wide range of anti-cancer agents by active extrusion of these molecules from neo-plastic cells. Significant interethnic variations in allele and genotype frequencies of MDR1 single nucleotide polymorphisms have been identified worldwide and there were no data available for the Egyptian population. The work was undertaken to assess the expression of MDR1 gene among Egyptian breast cancer patients. The genotype and allele frequency of MDR1 gene C3435T and C1236T polymorphisms were investigated as well as the possible correlation between these polymorphisms and the expression level of the gene. The expression level of MDR1 gene was assessed using reverse transcriptase-polymerase chain reaction. MDR1 gene C3435T and C1236T polymorphisms were genotyped using polymerase chain reaction restriction fragment length polymorphism. Results showed that 82.8% were positively expressing the MDR1 gene at diagnosis, while 17.2% were negatively expressing the MDR1 gene. As for the C3435T polymorphism, The CC genotype, heterozygous CT and mutant T-allele were found in 31%, 51.7%, and 17.3% of the patients, respectively. The heterozygous CT genotype was the most predominant genotype among the patients positively expressing the gene. As for the C1236T, all the patients were found to be of the same genotype. To the best of our knowledge, our study provides the first data available on the genotype and allele frequency of MDR1 gene C3435T and C1236T Polymorphisms in Egypt. Our results could serve as a basis for large-scale correlation studies on the relevance of these polymorphisms in cancer therapy in Egypt.

Optimization and Kinetic Study of a *Bacillus* Surfactin and its Possible Application for Soil Washing

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A biosurfactant was produced efficiently by *Bacillus* cells which were isolated from Egyptian oil-contaminated soil. The bacterial isolate was identified using classical and molecular techniques as *Bacillus* sp. Biotech1 (JN836728). The biosurfactant, produced constitutively, was identified as surfactin through MALD-TOF-MS technique. A sequential strategy was used to optimize surfactin production including the OVAT system, Plackett-Burman design, and Central Composite Design (CCD). CCD was applied to five variables: incubation temperature, kerosene concentration, yeast extract concentration, inoculum size, and incubation time in 54 trails (based on data derived from Plackett-Burman design). Statistical analysis of results showed that only linear (incubation time and inoculum size) and quadratic kerosene concentration had highly significant effect on surfactin production with regression coefficient 0.85. Solver function of Microsoft Excel was used to determine the optimum conditions concerning surfactin production. Grown 2x108 cells on Mckeen medium containing 0.62% kerosene, 0.062% yeast extract at 32.24ºC for 10.76 days were the optimum conditions that support maximum emulsification activity with predicted value 4.204. Verification of this statistical model was applied, where the experimental activity was 4.101 which represents 97.5% of the predicted value. Consequently, this model can be trusted to describe surfactin production by this strain. Emulsification activity was increased by a factor of 12 (due to applying this model) over its value using original Mckeen medium. Kinetic study was also carried out where surfactin production by this strain can be well described by the logistic model. The logistic model gave satisfactory representation of both biomass growth and surfactin production with average error lower than 1% and CMC 0.301 g/L. The produced surfactin was examined for its ability to wash oil artificially contaminated soil where the removal efficiency reached 67%. Data would encourage applying optimization of surfactin production on a large scale for its use in the field of soil bioremediation to obtain a green environment.

Recombinant Expression, Purification of L-Asparaginase-II from Thermotolrant *E. coli* Strain, and Evaluation of its Antiproliferative Activity

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Repeated use of L-asparaginase II enzyme, in the treatment of acute lymphoblastic leukemia, is commonly needed because of the enzyme's instability and relatively short half-life which leads to more serious side effects on patients. In this study, we report on the cloning and expression of L-asparaginase from a thermotolerant strain of *E. coli* (KH027) which, when isolated from camel manure, can grow at 45°C. Expression of recombinant asparaginase was conducted by fusing asparaginase gene to pelB leader sequence and 6His residues at the C-terminus under the inducible T7 promoter in DH5α cells. Induction of the cells with 0.1 mm isopropyl-β-D-thiogalactopyranoside (IPTG) at late log phase of growth resulted in 1.6-fold (2111 UI) higher to that obtained in early log phase induction (1319 UI) and 1.3 fold compared with mid log phase induction (1623 UI). The purified protein showed optimum activities at a temperature of 43°C and pH 6. The Km and Kcat parameters were 3.8 mm-1 and 2.92×103 s−1, respectively. The enzyme retained around 57% and 30% of its initial activity after 30 and 60 minutes of incubation at 50°C, respectively. Recombinant L-asparaginase was evaluated for its antiproliferative effect in the leukemia cell lines of RS4; 11 and HL60 after 96 and 72 hours of incubation. The doses of 100 µg/mL and time-response effect of 96 hours caused a reduction value of 50% in cell viability of RS4. However, cell viability of 50% in the leukemic cells HL-60 was noticed with a concentration of 200 µg/mL with an incubation period of 72 hours. *In vitro* antiproliferative results in the leukemia cell lines make further *in vivo* investigation one of our research priorities to increase the possibility of using this thermos table enzyme in leukemia therapy.

Mapping QTL Affecting Multifactorial Traits in Chicken

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Growth traits, and in particular the yield of muscle mass and the amount of fat deposited in the body, are the most important traits that influence the nutritional and economic values of chickens. Therefore, it is desirable for chicken breeders to know the genetic determinants of these traits to be used in the breeding program. The aims of this study are mapping genomic regions affecting several growth traits and estimating genetic effects of identified regions. Reciprocal F2 crosses (n=579) between extremely divergent inbred lines, New Hampshire and White Leghorn, were used to map Quantitative Trait Loci (QTL) for growth performance (body weight and body weight gain) and body compositions (muscle mass, carcass parts and fat deposit). All animals were genotyped at 123 marker loci covering 25 chromosomes. We mapped genomic regions on 22 chromosomes affecting 24 phenotypes. Linkage analysis provided evidence for highly significant QTL effects for growth performance and body compositions on GGA2, 4 and 27. The peak QTL positions for different traits were located on GGA2 between 33.10 and 112.41 Mb, on GGA4 between 75.24 and 79.39 Mb, and on GGA27 between 3.61 and 3.82 Mb. The distal region of GGA4 (42.01 Mb–88.41 Mb) showed the highest effects on all analysed phenotype. This region accounting for 4.6% to 40.2% of the phenotypic F2 variance of the corresponding affected traits. Additional genome-wide significant and highly significant QTL for different analysed traits were mapped on GGA1, 5, 7, 10, 11, 12, 15, and 26. For intramuscular fat content, a suggestive QTL was located on GGA14. Some loci have been reported in other studies. Other QTL effects were described for the first time. The difference between the parental lines and the highly significant QTL effects on GGA4 will further support fine mapping and candidate gene identification.

Transdifferentiation of Bone Marrow Mesenchymal Stem Cells into

Neural Cells via Cerebrospinal Fluid

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Recently, adult Bone Marrow-derived mesenchymal Stem Cells (BMSCs) have been reported to be able to not only differentiate into mesodermal cell types but also be reprogrammed to transdifferentiate into cells expressed endodermal and ectodermal genes. Thus, hypothetically, BMSCs could transdifferentiate into neuroectodermal cell types. Cerebrospinal Fluid (CSF) that fills and surrounds the Central Nervous System (CNS) contains electrolytes, proteins, sugars, special growth factors, and other signals. This microenvironment promotes the Neural Stem Cells (NSCs) commitment and differentiation into neurons and glial cells. This occurs in response to any injury or physiological changes. This study aims to induce BMSCs to transdifferentiate into neural-like cells, either neurons or glial cells, by using cerebrospinal fluid in vitro. BMSCs were aspirated from rabbit femur then isolated and cultured. Autologous CSF was added daily to the supplemented culture media. After that, we found dramatic morphological and biochemical modifications in the cultured cells. These cells were hypothesized to be neuronal-like and astrocyte-like cell. Different methods were used to verify our hypothesis. Cresyl violet stain revealed the Nissle bodies found in pyramidal cell bodies which were stained with the violet color. Silver impregnation depicted the cell bodies' processes with a black color. This indicated that they contained neurofilaments characteristics of axons and dendrites. Both of these results proved that BMSCs differentiated into neuronal cells. Also, BMSCs were differentiated into astrocytes which were verified by using Periodic Acid-Schiff (PAS) demonstrated glycogen granules, which frequently exist in astrocytes. Also, immunocytochemical examinations for Glial Fibrillic Acid Protein marker (GFAP) revealed a positive reaction indicating successful astrocytes differentiation. It was concluded that the induction of BMSCs with CSF mimics the strategy done in the CNS. CSF provided the essential niche for promoting the transdifferentiation of BMSCs into neural cells. These cells could hopefully help in treating acute and chronic neurodegenerative diseases.

Role of MIR-181A and MIR-146A in Regulation of NK Cell-Mediated Immune Response to Cancer

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Natural Killer (NK) cells are subset of bone marrow-derived lymphocytes of the innate immune system that constitutively expresses lytic machinery able to target and kill tumor cells independently from any previous activation. NK cells are also able to eliminate metastatic cells in the circulation. Therefore, NK cells are important mediators of anti-tumor immunity, limiting their growth and dissemination. The activation of NK cells is finely tuned by a delicate balance between signals delivered by activating and inhibitory surface receptors. NK cells control the expression of their surface activating and inhibitory receptors when challenged by cancer cells by a yet unclear mechanism. microRNAs (miRNAs) are small noncoding RNAs that post-transcriptionally regulate the levels of their targets mRNAs by inducing their degradation or blocking their translation and are therefore potential candidates for mediating this control process. In this paper, we are investigating the expression of miR-181a and miR-146a in NK cells isolated from peripheral blood of breast cancer patients using the gold standard RT-qPCR. The level of expression of miR-181a and miR-146a will be correlated to the level of expression of the NK cell activating receptors NKp30, NKp44 and NKp46. The level of expression of NK cell activating receptors are measured using flow cytometry. Expression of miR-181a was decreased tenfold on average in NK cells isolated from breast cancer patients compared to normal subjects (n=24, $P<0.0001$). However, miR-146a expression was not significantly different in NK cells isolated from breast cancer patients compared to normal subjects (n=24, $P=0.7361$).

Molecular Analysis of Antibiotic Resistance Genes in Clinical Isolates from Egyptian Hospitals

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The rapid emergence of microbial strains that are resistant to almost every existing antibiotic, often described as "superbugs", is a serious threat to human health, with the eventual risk of depletion of all antibiotic choices against some bacterial pathogens by the next decade. *Escherichia coli* (*E. coli*) is particularly known for its genomic plasticity and its subsequent ability to horizontally acquire novel genetic characters, including antibiotic resistance, especially in the hospital environment. Major outbreaks of Extended-Spectrum βeta Lactamase (ESBL)-producing *E. coli* (ESBLEC) have been reported in Egypt and other countries. Due to the difficulty to track the spread of antibiotic resistance contributed by closely related genes, this study was designed to investigate the outbreaks of ESBL among *E. coli* clinical isolates. One-hundred-and-eight clinical isolates were collected from the National Cancer Institute, Qasr El-Ainy, and Abo-El-Reesh Children's Hospital in Cairo, Egypt; their antibiotic sensitivity was screened by the Kirby-Bauer disk diffusion method. The antibiotic resistance phenotypes of those isolates were further quantified via calculation of the Minimum-Inhibitory Concentration (MIC) of major beta-lactam antibiotics against all the isolates. As a first step towards high-resolution resistance gene mapping, we used PCR primers to amplify two genes of the CTX family (CTX-m-1 and CTX-m-15), as well as the TEM and SHV families of beta-lactamases. Based on primary analysis of PCR results in the 108 isolates, we define correlation and differentiation patterns of different gene pairs. For example, more isolates have CTX-m-15 than CTX-m-1 genes (58 vs. 48), and 20% of the isolates had the three gene families. Future studies will focus on defining the most significant genetic biomarkers that can be used to track the rapid spread of multi-beta lactamase-resistant strains and correlate it with hospital antibiotic policies.

Enzymatic Neutralization of Venoms of Two Egyptian Vipers by Mango Kernel Methanol Extract

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Despite being rich in bioactive compounds, several million tons of mango kernels and peels, from several different sources, are wasted annually. The aim of this study is to investigate whether the mango (*Mangifera indica L.*) kernel has any neutralization activity against the main enzymes of the venoms of two clinically important Egyptian snakes: *Cerastes cerastes* and *Echis coloratus*; *Viperidae*. The methanolic extract of the Egyptian mango kernel (Hindi) was tested for its antioxidant and inhibitory activity of toxic enzymes like phospholipase A2 and proteases of the venoms of two Egyptian vipers. The extract effectively neutralized *in vitro* the enzymatic activities of proteases and phospholipase A2 of the venoms of *Cerastes cerastes* and *Echis coloratus* in a dose-dependent manner. The inhibitory effect of the proteases enzymes clearly supported on the SDS-PAGE. Additionally, the antioxidant capacity of the extract showed free radical scavenging effects dose dependently using the DPPH, ABTS, and phosphomolybdate methods. The results indicated that the powder of the kernel methanol extract may be used as a rich source of potential endogenous inhibitors of snake toxins and may help in improving the snakebite therapy.

Plants That Fight Cancer or *Trichothecium roseum* as a Potential Cancer (MCF-7) Preventive

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An increasing amount of cancer research is being directed towards the investigation of plant-derived anticancer compounds, many of which have been used in traditional herbal treatments for centuries. Advances in environment legislations and biodiversity conventions are expected to minimize and prevent the over-collection of medicinal plants for industry. The annual death toll from cancer is expected to rise to 17.5 million by 2050. This pushes our team to screen endophytic fungi for their ability to produce anticancer metabolites. A total number of 49 endophytic taxa from 8 plant species were isolated from different altitudes in Saint Catherine. *Stachybotrys chartarum* and *Trichothecium roseum* recovered from *Origanum syriacum* and *Achillea fragrantissima* plants were identified morphologically and confirmed by molecular technique which was carried out by comparing the 5.8S-ITS2 rDNA region sequence data deposited in GenBank. To explore the anticancer activity, metabolites of *T. roseum* and *S. chartarum* were tried against *Ehrlich ascites* carcinoma cells in-vivo in female Swiss albino mice when inoculated intraperitoneally (0.2 ml of 2×106 cells/mouse). The therapeutic effects of both EtOAc and aqueous extracts were evaluated on EAC-bearing mice Body Weight Gain (BWG), Tumor Volume (TV), Median Survival Time (MST), and percentage-Increased Life Span (%ILS). Moreover, their effects were tried *in vitro* on liver and kidney biochemical parameters. Tumor markers were also investigated. Both EtOAc and aqueous extracts of *T. roseum* significantly decreased BWG and TV but significantly increased MST and %ILS to 23–27 and 48–71% respectively. *Trichothecium roseum* extracts did not alter liver or kidney functions, but significantly reduced the tumor markers for breast cancer (CA 15.3), ovarian cancer (CA 12.5), pancreas cancer (CA 19.9), carcinoembryonic antigen (CEA) and Alpha-fetoprotein tumor marker (AFP). Results showed that fungal endobionts of medicinal plants are a significant potential and sustainable source of novel bioactive compounds.

Enhancing the Mechanical Properties of a Mandible Through Induced Bone Regeneration: An Experimental Study

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Tissue engineering/regenerative medicine provides a recent approach for assembling new bony tissue for the defective mandible. The present study was conducted to test the mechanical integrity of the mandible in which a critical size defect was regenerated through using bone morphogenic protein

2. Methods: Twelve adult male goat mandibles were categorized into three groups (of four goats each). Group A included normal mandibles, group B included mandibles with a critical size defect (2x3 cm) created at the mandibular angle, while group C included mandibles with a similar defect filled with regenerated bony tissue. In group C, a size-to-size scaffold made of biphasic ceramic was charged with platelet rich plasma and bone morphogenic protein 2, surgically fixed in place using plates, wires and screws and then explanted after six months. Mechanical testing of all mandibles was done using a three-point bending apparatus by applying a load on the anterior boundary of the designed defect, and the force required to make a break in the mandible was recorded in Newton (N) and compared among the three groups using the Mann-Whitney test.

Results: The median "force" required to break the mandible was significantly ($p<0.05$) higher in group A than in group B (678.20N and 206.25N, respectively). Mandibles with regenerated defects (Group C) required a significantly ($p<0.05$) higher "force" (1476.60N) as compared to both Groups A and B.

Conclusion: Inducing bone regeneration could be an efficient method for reconstructing critical size defects at the mandibular angle.

Heterologus Expression, Purification, and Characterization of Different Thermostable Carboxyl-Esterases (A) Obtained from Saudi Arabian Isolates

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A PCR-screening program was performed for the cloning of Carboxyl-Esterase A from thirty-one different *Geobacilli* isolates obtained from different localities using degenerate primers. Among these isolates, three isolates were selected: *Geobacillus caldovelox III*, *Geobacillus caldovelox IV*, and *G. thermodenitrificans XI*. The gene related to these selected isolates was cloned and successfully expressed in pCYTEX-P1 expression vector under lambda promoter. Upon induction the maximum yields (444, 610, and 292 u/L/min) of expressed proteins (~28 kD) were obtained after 4 hours, respectively. For one step purification using affinity chromatography technique, the recombinant proteins were modified by fusion of 6X-histidine to the C-terminal chain. The produced recombinant proteins were purified till unity using Fast Protein Liquid Chromatography technique (FPLC) and consequently, the purified proteins were characterized.

Jak2/Stat5a Overexpression Synergizes with Anti-Cancer Drugs to Inhibit Breast Cancer Steam Cell Populations that are responsible for Epithelial-Mesenchymal Transition, and Invasion Potential

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Epithelial-Mesenchymal Transition (EMT) is a key event in the tumor invasion process and poor prognosis of breast cancer which is the main leading cause of cancer-related deaths among women. EMT is associated with a high potential of cancer cells invasion and metastasis. Recently, a growing body of evidence from our laboratory is supporting the idea that human breast cancer can be considered as a stem cell disease and the metastatic cell state is strongly correlated to (EMT) and the CD44+/CD24- stem cell phenotype. We have recently uncovered novel evidence that signal transducers and activators of transcription 5a (Stat5a) and its Janus tyrosine kinase (Jak2) suppress early invasion and promote human breast cancer cells differentiation through inhibition of (EMT) dedifferentiation. In the present study, a comparative analysis of epithelial and mesenchymal marker expression was performed, using a panel of human breast cancer cell lines that display different phenotypic and differentiation patterns and expressing different levels of CD44+/CD24- (BCSC) population. Gene expression profiles of the tested cell lines were examined to identify a set of genes that could play a role in (EMT) and evaluated by RT-PCR and flow Cytometry. Our data showed that Jak2/Stat5a overexpressing cells synergizes with some commercial available anti-cancer drugs in apoptosis induction by activating capase-3 and inhibiting the expression of Bcl-2 in CD44+/CD24- (BCSC) population. Furthermore, the cell lines that overexpress Jak2/Stat5a and are pretreated with anti-cancer drugs inhibit (EMT) by blocking the expression of nuclear β-catenin, Vimentin, Slug, and Snail, and also decrease small populations of CD44+/CD24- (BCSC) invasion capacity by induction of E-cadherin expression, suggesting the blockade of signaling involved in early process of metastasis through elimination of cancer stem cell characteristics, which could be a new strategy to inhibit breast cancer metastasis.

Development of Transgenic Wheat Plants Tolerant to Abiotic Stress Using MDAR Gene

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Monodehydroascorbate Reductase (MDAR), an important enzyme of theascorbate-glutathione cycle, is involved in salt tolerance of plants through scavenging of Reactive Oxygen Species (ROS). In this study, a cDNA encoding AtMDAR1 gene from the *Arabidopsis* plant was introduced into wheat plants of Bobwhite 56 cultivar using biolistic gene gun to examine its role in salt tolerance. Two stable transgenic plants (MD5 and MD6) were selected *in vitro* in the second generation (T2). Overexpressing MDAR1 gene was selected *in vitro* using basta and confirmed by quantitative reverse-transcription PCR (qRT). The transgenic plant MD6 was predicted to possess two copies of the transgene, while the other transgenic plant MD5 was predicted to have four transgene integrations. The AtMDAR1 transcripts in transgenic wheat plants were higher than untransformed plants. The abundance of AtMDAR1 transcripts in transgenic plants MD5 and MD6 were approximately 1.75 and 1.65, respectively, times the amount found in non-transgenic plants. MD5 and MD6 plants, also, accumulated greater amounts of Ascorbic Acid (AsA) than the non-transgenic plants. They also showed tolerance to salt at germination stage under NaCl 200 mm concentration (11,600 ppm). In a greenhouse experiment, these transgenic plants showed more vigorous growth than the non-transgenic plants (Bobwhite56) at 200 mm NaCl. In a high salt enviroment, transgenic plants MD5 and MD6 had higher dry mass, shoot, and root length, and higher Tolerance index (Ti) in comparison to the non-transgenic plants. This suggested that the transgenic plants were more tolerant to salt stress and have potential for breeding salt-tolerant wheat.

Key Words: Wheat, MDAR, ROS, Bombardment, Transformation.

A Case-Control Study on the Association of Melatonin and MTNR1B gene rs#10830963 with Breast Cancer

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This study aims to figure out the role of melatonin and its receptor MTNR1B gene rs#10830963 polymorphism in breast cancer incidence, diagnosis, and prognosis.

Subjects and Methods: 43 breast cancer female patients and 45 seemingly healthy female pateints were included in this study. Restriction Fragment Length Polymorphism (RFLP)-PCR was used for amplification and genotyping of MTNR1B gene rs#10830963 polymorphism in whole blood. Serum melatonin levels were measured using a ready-for-use radioimmunoassay kit.

Results: For MTNR1B gene rs#10830963 polymorphism, the GG genotype frequency was significantly higher among cases (72.1%) than controls (13.3%), with diagnostic sensitivity (83.7%) and specificity (76.47%). The frequencies of CC and CG genotypes were significantly lower among cases (11.6% and 16.3%, respectively) than controls (44.4% and 42.2%, respectively). The presence of GG genotype significantly increases the risk for breast cancer incidence by about 21 times compared with the CC genotype. The GG genotype was significantly associated with larger tumor volume ($p=0.04$). Serum melatonin levels were significantly lower among breast cancer patients than controls. Females with serum melatonin levels $\leq$39.5 pg/ml are at significantly increased risk for breast cancer incidence by about 15 times more than females with levels>39.5 pg/ml.

Conclusion: The risk for breast cancer incidence can increase as the serum levels of melatonin decrease and in females who are homozygous for the G allele of rs#10830963. Moreover, The GG genotype was found to be associated with increased breast tumor volume which is one of the markers of poor breast cancer prognosis. To the best of our knowledge, this is the first study investigating the association of rs#10830963 polymorphism with the risk of breast cancer incidence.