Paper 1

Anti-inflammatory potential of *Vachalia nilotica* and *Calotropis gigentean* leaves against cold induced paw inflammation

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Abstract

Vachilia nilotica and *Calotropis gigentean* has therapeutic ramifications against infections due to various phytochemicals like flavonoids, alkaloids, tannins, and polyphenolic compounds. So current study was designed to investigate anti-inflammatory potential of polarity based extracts of leaves of *V. nilotica* and *C. gigentean* against ice induced cold injury in paws of albino rats of both gender (225- 250 grams) by keeping diclophenic sodium as positive control and checked paw size by vernier caliper after 1-10 days, followed by the histopathology of the skin of rats. Statistically analyzed results showed that ethyl acetate, chloroform and water extracts of *V. nilotica* leaves were more potent to reduce paw edema, particularly in male rats. This activity was due to homaline, hopromine, hoprominol and hopromalinol in leaves of this plant so current work can be used as basic work for the isolation of active compounds against cold induced injury.

Key words: Cold induced inflammation, *Vachalia* nilotica, *Calotropis gigantean*, Albino rats

Paper 2

Herbal antioxidants in drug development

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Abstract

Plants have been medicinally used from ancient times till today as a source for many pharmaceuticals. Although current pharmaceutical industry had been born from botanical medicine but synthetic strategies for drug discovery turned out to be standard. However, there's a turn down for new drug development in recent era with an expanding market for botanical therapeutics as drugs/ botanical drugs and dietary supplements. Mostly botanical therapeutics is originated from medicinal plants which have been cultured for bioactive components but with the passage of time, phytochemical composition of plants has been changed due to environmental changes, which lead to the colonization of edible parts of plants for more content and better adapted bioactive compounds with reduced content of others, which are making plants as important source of therapeutic compounds with infinite biosynthetic capacity. A principal advantage of botanicals is their intricate composition which consists of cluster of related compounds having numerous activities that interact for a greater total activity. It is well known that free radicals definitively damage DNA/RNA, carbohydrates, unsaturated lipids, proteins, and micronutrients such as carotenoids (alpha and beta carotene, lycopene), vitamins A, B_6, B_{12}, and folates which are defended through enzymes and body's natural antioxidants. These antioxidants complement each other rather than compete with one another like carotenoids interact with vitamins E and C and phenols and bioflavonoids potentiate vitamin antioxidants e.g. rutin potentiates vitamins C and E to produce a more potent radical scavenging action. Moreover plant based antioxidant factors are based upon constituent nutrients and non-vitamin or mineral substances. So, in addition to alpha-tocopherol, ascorbate, carotenoids, and zinc, plant-based medicines may contain flavonoids, polyphenols, flavoproteins, thiols, retinoids, carbohydrates, trace metals, terpenes, tocopherols and degradation products of glucosinolates (isothiocyanates, indoles and dithiothiols), glutathione, uric acid, ubiquinol-10, vitamins A, C, and E, silymarin, triterpene saponin and others.

Key words: Antioxidants, Pharmaceutical industry, Plants

Paper 3

Natural product-based drug development; An evergreen tool as emerging therapeutic agents

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ABSTRACTS

Discovery and development of drug is collectively a complete process to identify a new drug to bring it to market. Drug discovery may involve screening and identification of chemical libraries and active ingredients respectively either from a natural remedy or from a design on the basis of specific target while drug development includes *in vitro* and *in vivo* studies on microorganisms and animals, followed by clinical trials and regulatory approvals. In current scenario, it is done by DNA-encoded library technologies for the success of selection, genome studies for biosynthetic gene clusters to expose mysterious metabolic potentials, human-on-a-chip model (based on human hepatocytes and adipose tissue chambers) for the identification of contributing metabolic factors for the development, progression, and assessment of liver disease therapeutics, development of pro-drugs by radiotherapy, investigation of dietary agents on different drug targets through *in silico* studies, fragment-based or fragment pharmacophore-based drug design, identification of inhibition mechanism of viral proteases by metabolites and their derivatives, crowd sourcing drug discovery for pandemics, protein targeting with SAF (er)

electrophiles as chemical proteomic probes, trashing transcription and oligonucleotide designing for personalized treatment along with many more. Each era of drug discovery to development always faces its toughest challenges due to increased pressures with respect to overheads and sustainability, natural targets and chemical matters for designing to modulate disease and all these issues are becoming even more complex with the passage of time. Although novel drugs have a revolutionary role on human health in last century, but there are considerable unmet medical needs as diseases of ageing, oncology, antibacterial and antiviral resistance and cardiometabolic diseases are continuously posing a substantial burden on society and this is due to the gap between elementary scholarly and functional industrial research which is the need of time to ensure real-world applications to bridge well-defined mutual academia–industry projects by encouraging better communication between the scientists involved in them.

Keywords; Medicinal plants, drug discovery, active compounds

Paper 4

Hepatoprotective Role of Curcumin and Honey Against Amikacin Induced Liver Injury in Mice Model

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ABSTRACT

Liver in the most important internal organ involved in many physiological functions which withholds the body through traumas. Liver pathologies are concerned with toxins. Usage of medicines is not an ideal approach because of their side effects. The purpose of the study was to examine hepatoprotective effects of interaperitoneally induced curcumin (100mg/ Kg b.w and 200mg/ Kg b.w) in combination with honey (500mg/Kg b.w) in male albino mice (100-150g) against amikacin (35mg/Kg b.w) induced hepatotoxicity for seven days, followed by the evaluation of ALT, AST, Albumin, total protein and bilirubin from serum and histopathological studies of hepatic tissue. Statistically analyzed results showed sever condition of fibrosis to hepatic tissues due to the administration of Amikacin. Albumin was significantly reduced due to the administration of curcumin and honey ($P<0.05$), while effects on total Bilirubin, ALT, AST and Total protein were non-significant when100 mg/Kg b.w of curcumin and 500 mg/ Kg b.w of honey were administered. Current work conclude that honey in amount of 500mg/Kg b.w along with curcumin (100mg/Kg b.w) has a considerable effect to cure hepatic cellular damage while curcumin has potential to reduce oxidative stress due to amikacin. Moreover curcumin and honey showed synergistic effects for the treatment of hepatic injury. Moreover altered concentrations of honey and curcumin for treating toxicological pathology of liver can be performed in future, along with enhancement in the treatment quality and isolation of hepatoprotective compounds from honey, curcumin or their combination to use them in pharmaceutical industry.

Keywords: Curcumin, Honey, Amikacin, hepatic tissues

ANTIBACTERIAL ACTIVITY OF POLARITY BASED EXTRACTS OF *CHLORELLA SOROKINIANA* AND *DESMODESMUS INSIGNIS* AGAINST HUMAN PATHOGENIC CLINICAL ISOLATES

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ABSTRACT

Bacterial resistance to is emerging throughout the world due to modified genome and changing environmenatal condiations that it seems to be a diaster to destroy the nations all around the world. So urgent and rapid measures are need of hour by clinicians, microbiologist and chemists to search a new source for better antibacterial or antibiotic production . Microalgae can be a new arena against bacterial infections. So currernt work has been done to check antibacterial activity polarity (n-Hexane, Ethyl acetate, Chlorofrom, Ethanol and water) and temperature (25- 60 ºC) based extracts of *Chlorella sorokiniana* and *Desmodesmus insignis* by agar well diffusion method at 1.0 mg/ml against clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumanii*, *Escherichia coli* and *Klebsiella pneumoniae*, from urine and sputum samples of human, followed by the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of antibacterial extracts by serial diution and streak plate methods respectively. Statistically analyzed results showed that at at 45 ºC, n-Hexane extract of *C. Sorokiniana* was more effective against *A. baumanni* while ethyl acetate and chloroform extracts were highly effective against *P. aeruginosa*. Moreover fresh extract of ethyl acetate of *C. sorokiniana* was also effective against *P. aeruginosa.* MIC of all extracts was found to be 50 ug/ ml while MBC, which lead to thin bacterial growth, was 25 ug/ ml by all inhibitory extracts of *C. Sorokiniana* and *D. Insignis*. Both algal strains were strongly antibacterial against tested pathogens but *C. Sorokiniana* showed much promising resutls than other. Zones of inhibition can be enlarged on increased concentrations of microalgal extracts. Current work can be extended up to the isoaltion of specific bioactive compound (s) and mechanisms of their antibacterial action so that this work could make a base for the synthesis of antibiotics by pharmaceutical industry.

Key words: Antibacterial activity, Polarity based microalgae extracts, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

Pharmaceutical Materials Used For Drug Development Based On Bioactive Carbohydrates

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Abstract

Bioactive compounds from plants, foods or other natural sources represent a very important tool to improve health. There is currently a big interest in the study of bioactive compounds, extracts, and new ingredients from natural sources in order to produce pharmaceuticals, functional foods, nutraceuticals, botanicals, and dietary supplements.

Certain non-nutritive bioactive compounds present in foods and plants, known as phytochemicals (polyphenols, terpenes, alkaloids), offer a wide chemical diversity responsible for a wide range of pharmacological and biological properties; additionally, other bioactive compounds can be macro- or micronutrients with additional health benefits beyond their nutritional properties (omega 3 and 6 fatty acids, dietary fiber, vitamins, amino acids, etc).

Bioactive carbohydrates like glucose, fructose, sorbitol, mannitol, ribose, deoxyribose, hyaluronic acid and glycoproteins have wide range of biological activities and help human body to perform proper function for survival . Among all mannitol is consider as more active compound both physiology and chemically

Mannitol is diuretic and renal diagnostic aid which is related to sorbitol. It is also **called D-mannitol, Osmitrol, or mannite. It is a naturally occurring alcohol found in vegetables an**d fruits. It is the most effective and safe medicine in the health system and listed on the World Health Organization's List of Essential Medicines. It is a prescribed drug used to reduce elevated pressure in the brain. Briefly mannitol ($C_6H_{14}O_6$)

- It is used in cyanide poisoning as an antidote.
- It is used to measure extracellular body fluid as well as to measure the renal glomerular filtration rate.
- Used as treatment of calciphylaxis in hemodialysis.
- It is used during cardiopulmonary bypass.
- It is used to decrease pressure in the eyes, such as in the case of glaucoma
- It is used as adjuvant in vaccine preparation

Paper 7

Screening of Thiourea Derivatives as Potential Inhibitors

of Sars-Cov-2

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Abstract

The new corona virus (SARS-CoV-2) that appeared towards the end of 2019 is a global public health issue due to its high morbidity and fatality rates. SARS-CoV-2 enters the cell via attaching to the host cell's ACE2 receptor via the Receptor Binding Domain (RBD) of spike protein. It was recently discovered that anti-covid-19 medications interact with multidrug resistant ABC transporters, namely ABCB1. This causes serious difficulties in COVID patients owing to increased drug extrusion, which affects medication dose. In the current investigation, a range of thiourea compounds and polyherbal formulations were tested as possible SARS-CoV-2 inhibitors by targeting the attachment of spike protein (RBD) to ACE2. In addition, we investigated the interaction of thiourea derivatives with the human ABCB1 transporter in an overexpressing cell line. Results indicated that compound BB-IV-46 showed strong impairment of RBD-ACE2 attachment followed by BB-IV-34, BB-V-21 and BB-IV-60 by showing percentage inhibition of 99%± 0.016, 94.01%±0.001, 87.81%±0.002 and 82.06%±0.006 respectively relative to positive control. The same compounds interact with ABCB1 and inhibit cell proliferation of P-gp overexpressing cell line with IC50 value of 6.19±0.06, 0.5941±0.007574, 1.575±0.012587 and 4.484±0.007574 respectively. Furthermore, MD simulations followed by binding free energy analysis were carried out to explore the binding interaction of thiourea compounds BB-IV-46 and BB-V-19 to RBD region of spike protein of SARS-CoV-2. The results confirmed that compound BB-IV-46 interacts strongly with RBD with significant binding energy (-127.0 kJ/mol) while BB-V-19; the inactive compound with percentage inhibition of 22.35%± 0.002 interacts weakly with a small binding energy (-29.30 kJ/mol) as compared to the active compound Our research also focuses on RBD residues that interact with active thiourea derivatives. However, further pharmacokinetic and pharmacodynamicresearch are required before proceeding to clinical trials.

Paper 8

Evaluation of Single Neucleotide Polymorphism In MDR ABCC1 Gene In Diabetic Population Of Khyber Pakhtunkhwa, Pakistan

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Abstract

ABCC1 is a multidrug resistance ATP-Binding cassette transporter commonly known as multidrug resistance-associated protein 1 (MRP1). It is a full-length transporter and is located on chromosome number 16p13.1. Physiologically, it exports xenobiotics and toxins out of the cell, thereby providing a shielding mechanism for the cell. Under stressed conditions, ABCC1 becomes overexpressed, resulting in an efflux of drugs and creating a hurdle in the treatment of various diseases. Additionally, in the diabetic condition, it plays an important role in the development of endothelial dysfunction and reactive oxygen species (ROS). Several studies have reported the importance of the ABCC1 polymorphism in cancer patients in different populations. However, studies related to ABCC1 polymorphism in diabetic patients are rare, especially in Pakistan. Therefore, the present study was conducted to find genetic variations in the ABCC1 gene (exon 22) in a diabetic population (n=100) of Mardan, Pakistan. PCR-SSCP analysis followed by sequencing revealed mutations in 06 samples followed by sequencing. Results identified 9 novel genetic variations in diabetic subjects. Among these, 7 were found to be non-synonymous, 1 synonymous, and 1 intronic variant. In addition, rs3851716 (A1009G) has also been identified as a reported variant in dbSNP and the 1000 Genome project. The sequence homology-based webservers, PROVEAN (protein variation effect analyzer) and SNP-nexus were used to predict the damaging effects of 9 variants on protein function. The PROVEAN analysis of 9 variants characterized 3 variants as deleterious and 3 variants as neutral. The SIFT algorithm predicted 4 variants damaging and 3 variants tolerated. A cumulative PROVEAN, and SIFT analyses predicted 3 nsSNPs (16,16205244,G,C, 16,16205343,T,A, and 16,16205415,T,A) as “deleterious/damaging”. SNP-nexus predicted 1 variant as synonymous and 1 in the intronic region; these 2 were excluded from further study. This study for the very first time reported genetic screening of the ABCC1 gene and its effect on

structural modifications in proteins from District Mardan, Khyber Pakhtunkhwa, Pakistan that might be helpful in the development of anti-diabetic drugs by targeting the ABCC1 transporter.

Paper 9

Natural product-based drug development; An evergreen tool as emerging therapeutic agents

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Abstract

Drug discovery and development together are the complete process of identifying a new drug and bringing it to market. Discovery may involve screening of chemical libraries, identification of the active ingredient from a natural remedy or design resulting from an understanding of the target. Development includes studies on microorganisms and animals, clinical trials and ultimately regulatory approval. Currently it is accomplished by DNA-encoded library technologies (DELT) for the effectiveness of screening, genome mining for biosynthetic gene clusters for unveiling cryptic metabolic potential, human-on-a-chip model which is composed of human hepatocytes and adipose tissue chambers for modeling the metabolic contributing factors for liver disease development, progression, and evaluation of its therapeutics, Switching on prodrugs using radiotherapy, analysis of dietary agents on different drug targets through *in silico* studies, Fragment-based/ fragment pharmacophore based drug design, identification of Inhibition mechanism of viral proteases by secondary/ primary metabolites and their derivatives, Crowd sourcing drug discovery for pandemics, Protein targeting with SAF(er) electrophiles as chemical proteomic probes, Trashing transcription, Oligonucleotide designed for ultimate personalized treatment and many more. The world of drug discovery and development is facing its toughest challenges in a generation. Along with increasing pressures associated with costs and sustainability, the biological targets and chemical matter designed to modulate disease are typically becoming ever more complex. And despite the revolutionary role that novel drugs have had on human health in the past century, significant unmet medical needs remain. Diseases of ageing, oncology, antibacterial and antiviral resistance, as well as a host of cardiometabolic diseases, continue to pose a substantial burden on society. It's all because of the gap between fundamental academic research and the applied industrial research that is necessary to ensure real-world applications, which can be bridged by engaging in well-defined collaborative academia–industry projects and fostering better communication between the scientists involved in them.

Keywords; Medicinal plants, drug discovery, active compounds

Paper 10

P.41: In Silico Studies Of The Abietadiene With Potential Serine Protease Inhibitory, Antithrombin, And Antiplasmin Activities For Treating Thrombotic Disorders

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Abstract

Cold weather induced injuries are becoming more prevalent as humans are becoming more exposed to subzero temperatures due to increasing tourism up north, military expeditions, and increased cold storages. Frostbite is one of the prominent cold weather injuries and can lead to devastating consequences such as amputation of the extremities. Although rate of amputation due to frostbite has decreased recently due to thrombolytic therapy but this new regimen comes with a price as well which include symptoms such as profuse gastrointestinal bleeding in patients. Plants are a great source of medications and have the advantage of being relatively safer. Statistically analyzed results of psychrophytes against dry-ice induced frostbite on plantar surfaces of Albino Wistar rats showed best results from the extracts of *P. roxburghii*. *In silico* validation of the results through molecular docking on CB Dock of secondary metabolites of *P. roxburghii*with modulators of anticoagulant pathway showed that best docking structures were obtained with abietadiene and antiplasmin (-8.1kcal/mol) and abietadiene and antithrombin III (-6.1 kcal/mol) which showed promising results in molecular device simulation (MDS) studies. Current work can be used as tool for the formulation of synthetic compound against thrombotic disorders.

Key Words: *In Silico*, Abietadiene, Antithrombin, Antiplasmin, Thrombotic Disorders

Paper 12

P.1 Anti-SARS-CoV2 Drug Development: Thiourea derivative BB707 (herbal formulation) as Prototype

Mohammad Assad, DrZahidaParveen, AkifKhurshid, DrBeenishKhurshid, DrSaira Farman, Department of Biochemistry, AWKUM

Abstract

The novel corona virus (SARS-CoV-2) that emerged at the end of year 2019 is considered as global public health concern, since it causes high morbidity and mortality. SARS-CoV-2 attaches to ACE2 receptor of the host cell through the Receptor Binding Domain (RBD) of spike protein thus gaining entry into the cell. Recently it has been reported that anti-covid-19 drugs interact with multidrug resistance ABC transporter, particularly ABCB1. This leads to severe complications in COVID patients due to higher extrusion of drug affecting the drug dosage. In current study, a series of thiourea derivatives and polyherbal formulation was screened as potential inhibitors against SARS-CoV-2 by targeting the attachment of spike protein (RBD) with ACE2. We also explored the interaction of thiourea derivatives with human ABCB1 transporter in overexpressing cell line. Results indicated that compound BB-IV-46 showed strong impairment of RBD-ACE2 attachment followed by BB-IV-34, BB-V-21 and BB-IV-60 by showing percentage inhibition of 99%± 0.016, 94.01%±0.001, 87.81%±0.002 and 82.06%±0.006 respectively relative to positive control.

The same compounds interact with ABCB1 and inhibit cell proliferation of P-gp overexpressing cell line with IC50 value of 6.19±0.06, 0.5941±0.007574, 1.575±0.012587 and 4.484±0.007574 respectively. Furthermore, MD simulations followed by binding free energy analysis were carried out to explore the binding interaction of thiourea compounds BB-IV-46 and BB-V-19 to RBD region of spike protein of SARS-CoV-2. The results confirmed that compound BB-IV-46 interacts strongly with RBD with significant binding energy (-127.0 kJ/mol) while BB-V-19; the inactive compound with percentage inhibition of 22.35%± 0.002 interacts weakly with a small binding energy (-29.30 kJ/mol) as compared to the active compound. Our study also highlights interacting residues of RBD with active thiourea derivative. However further pharmacokinetic and pharmacodynamics studies are needed to be done before taking it to clinical trials.