Summary and Conclusion
Cancer is a dreaded disease for which several lines of treatment have been developed. Chemotherapy is one of the most widely used modes of cancer treatment. Administration of most of the chemotherapeutic drugs leads to toxic side effects, as these drugs act for their cytotoxic action. In most of the cases severe toxicity of chemotherapeutic drugs make the life of the cancer patient miserable. Cyclophosphamide (CP) is a widely used chemotherapeutic drug. It metabolizes via cytochrome P450 (CYP 450) enzymes to its active metabolites acrolein and phosphoramid mustard. These toxic metabolites cause a wide array of toxic effects including, nausea, vomiting, immunosuppression and biochemical toxicity viz. weakening of antioxidant system and disruption of drug metabolizing enzymes. The toxicity related to cyclophosphamide becomes severe due to tumour burden in advanced stages of cancer. Therefore, it is important to test some agents/drugs which could be effective in reducing toxicity of cyclophosphamide not only in normal animals but also in tumour bearing animals. In the present study, such an attempt has been made to test compound Unani preparations against cyclophosphamide toxicity in the animals bearing Ehrlich’s ascites tumour.

Ehrlich's ascites tumour is a robust tumour model used widely for study of anticancer activity of synthetic chemicals, herbal extract and natural compounds with anticancer potential. It has been observed that when an Ehrlich's ascites tumour bearing animal is treated with cyclophosphamide,
the toxic effects are magnified. In recent times, many herbal drugs and their products are being used for treatment of diseases including cancer and for mitigating the toxic side effects of drugs and chemicals. The herbal drugs include single drugs, their active ingredients and compound herbal formulations, which are being used in traditional systems of medicine viz. Ayurveda, Siddha, and Unani-tib for different target organs and systems for a long time. Now, those drugs are being tested and explored for their efficacies in treating cancer and mitigating the toxic side effects related to cancer chemotherapy.

In Unani system of medicine like Ayurveda drug preparation is based on traditionally established therapeutic efficacy of herbal extracts or minerals. No doubt, in some cases animal products are also used as ingredients. Jawarish amla sada (JAS) and Habbe khabuls hadid (HKH) are two compound herbal formulations used in the Unani system of medicine as liver tonic and stomachic. While JAS contains significant amount of antioxidant herb amla (Emblica officinalis) HKH contains another important herb extract of methi or fenugreek (Trigonella foenum-graceum). Independently, huge amount of literature is available for various therapeutic potential of these two herbs. We have studied the modulatory effect of these two herbal drugs in mitigating the toxic effects of cyclophosphamide in tumour bearing mice.

Many investigators have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human
diseases like aging process. Numerous plant constituents have proven to show free radical scavenging or are antioxidants itself.

In order to test the antioxidant activity of Unani preparations *per se* in vitro assays based on DPPH* and ABTS** radical scavenging activity were performed. It was observed that both the Unani drugs (JAS and HKH) have in vitro antioxidant activity. Among the two, JAS appears to be more promising in scavenging ABTS** radicals. Unani herbal preparations which contain a mixture of medicinal herbs are good free radical scavengers thereby proving to be effective in reducing reactive oxygen species (ROS) related damage. Then we tested as to whether these drugs provide any protection to CP-induced disruption of drug metabolizing enzymes of liver and kidney or not. Drug/xenobiotics metabolizing enzymes of liver, in particular pay an important role in activation and deactivation of drugs and chemicals. Liver microsomal enzymes metabolize the drugs and chemicals to their active metabolites which then binds with the phase II enzymes present in the cytosol which facilitated their excretion. CYP 450 dependent enzymes of phase I and glutathione S-transferases (GST) of phase II drug metabolizing system work in unison facilitating generally detoxification of drugs and xenobiotics. However, their disruption or malfunctioning may have serious consequences. Herbal extract and drugs made out of them can affect drug metabolizing enzymes. In this study it was observed that both JAS and HKH have modulatory effect on drug metabolizing enzymes which were adversely affected by either CP treatment or EAT cell challenge. It has been observed in previous studies that tumour challenge not only affects the immune functions but also metabolic vital pathways. Disruption of phase II enzymes such as GST which contribute...
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to detoxification of endogenous and xenobiotic metabolites may be undesirable consequence during tumour development. Abnormal functioning of phase II enzymes may also contribute to accumulation of toxic metabolites of chemotherapeutic drugs thereby increasing toxic burden in the body. Both the Unani preparations positively modulated the disrupting effect of CP as well as tumour cell challenge on CYP 450 and GST. CP and other cytotoxic anticancer drugs severely affect the antioxidant profile. Disrupting effect of these drugs, in particular on reduced glutathione (GSH), which is the one of the most abundant form of free antioxidants of body, may aggravate the body’s defense against oxidative stress induced by various endogenous and exogenous factors. Adverse effect of CP on antioxidants and its oxidative stress-inducing effect are well documented in previous studies. In this study we observed that challenge of EAT cell resulted in reduction of antioxidants in liver and kidney and also increase in oxidative stress. When animals with CP or EAT cell challenge were treated with Unani herbal formulations the depleted antioxidants were restored. JAS and HKF also abrogated enhanced level of lipid peroxidation (LPO) in liver and kidney. LPO is responsible for cell membrane damage. All important antioxidant enzymes such as catalase (CAT) glutathione peroxidase (GPx) and glutathione reductase (GR) were positively modulated by Unani herbal formulations. Effect of these herbal preparations on GSH was in particular remarkable. Efficacy of these drugs was observed in normal as well as tumour bearing mice. Protective and restorative effects of these Unani drugs on antioxidants, particularly GSH offer promising role for these drugs as supplements along with chemotherapeutic drugs. Not only on antioxidants, these drugs also showed immunomodulatory effect.
Cyclophosphamide is a known immunosuppressive agent. Because of this property CP is even used in patients as an immunosuppressant to facilitate allograft implantation. CP in particular is toxic to humoral immune system. High does cause B cell knock down. CP treatment resulted in decreased plaque forming cell (PFC) counts in PFC assay. PFC assay is a robust assay of humoral immune function assessment. It provides measurable indication of primary antibody response. While effect of both the Unani preparations on relative organ weight was not so remarkable, effect on depressed PFC response was significant. Such effect was observed in normal as well tumour bearing mice. It was observed that CP treatment significantly depleted the cellularity of thymus and spleen in normal animals. Both the Unani drugs although caused some improvement in organ cellularity, the effect was not significant at the dose levels used in the present study. It may be possible that the doses used were not so effective in causing improvement in cell counts of the lymphoid organs. The hematotoxic effects of CP were also prevented by JAS and HKH. There are studies showing suppression of immune functions (both specific and non-specific) during tumour growth. The use of cytotoxic drugs such as CP aggravates immunosuppression state, as these drugs cause their own immunosuppressive effect by their inherent cytotoxicity to immune system cells. In this regard, the modulatory effects of Unani formulations on humoral immune functions are promising and needs further validation involving study of subsets of immunocytes. Although both the Unani formulations modulated some tumour-related parameters viz., mean survival time, tumour cell number, and tumour volume, the effect was not remarkable and further
studies may be required to prove efficacy of these herbal Unani formulations are anti-cancer drugs. The modulatory effect on these parameters might be a general tonic effect and might be transitory nature. EAT is an aggressive form of tumour which grow rapidly. It also requires very small initial inoculums to establish itself in host. Therefore, its control may be difficult.

The present studies are just indicators that Unani herbal formulations have potential to modulate antioxidants, enhance immune response and thus provide some relief to cancer patients. Regarding Unani drugs, there is lopsidedness in experimental studies when comparison is made with Ayurvedic drugs. More number of Ayurvedic drugs have been scientifically validated for their efficacy. Basically, there is huge similarity between two systems as far as preparation of formulations is concerned. Jawarish amla sada and Habbe khabsul hadid are herb based drugs. Some herbal extracts present in these Unani drugs are also present in several Ayurvedic drugs (Rasayanas). Amla and methi are two such examples. The traditional and folklore medicine system (including Unani-tib) work on the holistic approach whereby it is envisaged that the whole extracts of plants or combinations of different extracts with medicinal efficacy have higher therapeutic efficacy compared to the purified compounds. Therefore, it can be concluded pretreatment of patients, undergoing CP treatment, with herbal Unani preparations and other compound herbal drugs may restore the compromised redox balance and thereby minimize the deleterious effect associated with chemotherapy.
The studies reported in this thesis were conducted with limited scope. However, findings are encouraging and may require further validation with large groups and clinical validation. Although several lines of cancer treatments have been developed in recent years, chemotherapy is still one of the most reliable treatment lines for cancer control. Therefore, it is expected that use of CP and other chemotherapeutic drugs will continue to increase and so there will be need to prevent toxic side effects of these drugs. In this direction, drugs from traditional medicine system with tonic and adaptogenic effects hold promise of relief as complementary line of treatment.