SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

Oxygen free radicals, or more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products (oxidants) of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living system.

Overproduction of ROS results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures including lipids, proteins and DNA. Oxidative stress has been implicated in various pathological conditions including cancer, cardiovascular diseases, rheumatoid arthritis, diabetes and liver diseases

Antioxidants help in counteracting the activities of the oxidants. They are the substances that delay or prevent oxidation of cellular oxidizable substrates either by scavenging the ROS or by preventing the generation of ROS. Antioxidants are ubiquitous in natural products. Research is going on throughout the world to identify compounds with high antioxidant potential and low side effects.

Many plants and herbs have been identified as a source of antioxidants. Herbal plants have been used as a common source of medicine throughout the world and they form a major part of the traditional systems of medicine like Ayurveda, Siddha and Unani. Most of the therapies involve the use of plant extracts or their active components.

It is well known that the consumption of fruits and vegetables is essential for the maintenance of normal health in human beings. Some of the vegetables are treated as weeds in different parts of the world and as indigenous traditional vegetable in others. Such is the case of the *Solanum* species related to Blacknightshade (*Solanum nigrum* L.)

The *Solanum nigrum* L. and related species are widely used as leafy herbs and vegetables, as a source of fruit and for various medicinal purposes. The *Solanum nigrum* plants have been used in many Ayurvedic medicines. In spite of known uses in traditional medicines, documented evidence is not available on their possible mechanism of action.

The present study, therefore, aimed to probe into the antioxidant and anticancer effects of two varieties of *Solanum nigrum* leaves, one bearing the black berries (BBL) and the other one bearing the red berries (RBL) under the *in vitro*, *ex vivo* and *in vivo* conditions.

In the first phase of the study, two varieties of *Solanum nigrum* leaves were analysed for their antioxidant contents. Fresh leaves were analysed for their antioxidant enzymes (SOD, CAT, Px, GPx, GR, G6PD, AAO and PPO), non-enzymic antioxidants (ascorbate, α-tocopherol, total carotenoids, reduced glutathione, chlorophyll and phenols) and antioxidant minerals (Cu, Zn, Mn and Se).

Analysis of the results revealed that BBL exhibited higher activities of SOD, CAT and PPO while the RBL showed greater activities of Px, GPx, GR and G6PD. Significant difference was not observed in AAO activity. Among the non-enzymic contents, BBL was found to be richer source of tocopherol, total carotenoids and total phenols whereas RBL was richer in glutathione content. Ascorbate level was more in RBL but there was no significant difference between the two varieties. Thus, the first phase of the study iterated the antioxidant potential of the leaves.

In the second phase, initially, the radical and non-radical (DPPH, $SO^{\bullet}$, H_2O_2, $^{\bullet}OH$ and NO) oxidants scavenging activities were analysed. Both BBL and RBL were found to possess better scavenging activity on all these oxidants except NO. Both BBL and RBL exhibited negligible inhibitory activity on NO generation without any significant difference between them. BBL exerted a better scavenging activity than RBL. The oxidant scavenging activity of the two varieties of *Solanum*

nigrum is probably due to the presence of the various enzymic and non-enzymic antioxidants.

The ultimate cellular targets of the oxidative damage are the macromolecules, especially the lipids and DNA. Therefore, in the next step, to understand the antioxidant/ protective effect of the BBL and RBL, various sources of lipid and DNA molecules were subjected to oxidative stress were analysed.

Oxidative damage to lipid molecules causes lipid peroxidation. The extent of lipid peroxidation was effectively inhibited by both BBL and RBL extracts and comparatively BBL was found to be more effective in inhibiting the lipid peroxidation under conditions of oxidative stress induced in liver homogenate.

The protective effect of the BBL and RBL on DNA was analysed using various sources of DNA like viral (λphage), bacterial (pUC 18 plasmid), animal (herring sperm) and human (oral carcinoma cells) subjected to H_2O_2 induced oxidative stress.The extent of DNA damage was reduced in the purified DNA samples and the number of comet bearing cells was also found to be decreased with BBL and RBL treatments, while the BBL was found to exhibit a better protective effect against H_2O_2 induced oxidative stress.

The antioxidant response of BBL and RBL extracts were further analysed in the *in vivo* simulated *in vitro* system, the liver slices, in the presence and absence of oxidative stress (CCl_4). The activities of enzymic antioxidants (SOD, CAT, GPx, GST, GR and G6PD), non-enzymic antioxidants (Ascorbate, Vitamin E, Vitamin A and reduced glutathione) and lipid peroxidation (LPO) were analysed in the slices.

Exposure of CCl_4 caused a significant decrease in all the antioxidant contents and an elevation in lipid peroxidation. Co-administration of extracts, improved the antioxidant status in a differential manner in the liver slices. The activities of SOD and CAT were elevated by BBL to a higher extent while RBL was found to evoke the activities of glutathione dependent and related enzymes to a higher extent. The higher levels of vitamins E and A were found to occur in BBL treated groups whereas RBL treated group contained more GSH content. Vitamin C

level was considerably elevated without much difference between the two varieties was observed. Both BBL and RBL exerted a considerable level of inhibition but comparatively, BBL was found to exhibit a better inhibitory activity than RBL in this respect.

These observations suggest an interesting possibility in the mechanism of action of the two extracts. The differential picture in the responses shows that the leaves exert their antioxidant effects by different biochemical mechanisms. It can be suggested, from our results, that RBL extract acts via a glutathione dependent mechanism, while BBL follows a different route. This suggestion is validated by the observation that all the glutathione, dependent components (GPx, GST, GR and GSH) respond more significantly to RBL administration than to BBL. However BBL was found to evoke a better total antioxidant response than RBL.

Having established the antioxidant activity of BBL and RBL extracts in various *in vitro* systems, the cytotoxic activity of the extracts on KB (oral carcinoma) cells was analysed by the MTT assay. An effective cytotoxic effect was exhibited by both BBL and RBL extracts, with RBL showing better cytotoxic effect on cancer cell line, suggesting their anticancer activity.

The results obtained thus far, revealed the antioxidant and cytotoxic activities of both BBL and RBL extracts. These properties were further confirmed by using various *in vivo* models, because of the presence of various factors of the living system as well as the various other components of the extracts which may interfere with the above properties within the living system.Therefore, the third phase included the study of antioxidant and anticancer activities in rats and mice respectively.

The oxidative stress was induced in rats with the ethanol–CCl_4 system. Oxidative stress caused a significant elevation in the liver function marker enzymes and lipid profile in serum and an increase in hepatic cytochrome b_5 and cytochrome P450 activity was decreased in ethanol-CCl_4 treated animals. A marked decrease in antioxidant contents and an increase in lipid peroxidation products were observed in the liver tissues of oxidatively stressed (ethanol-CCl_4) animals.

The treatment with the extracts of BBL and RBL brought down the levels of liver function marker enzymes and the lipid contents in serum. A decrease in the activities of hepatic cytochrome b_5 and cytochrome P450 was observed in BBL and RBL extract treated groups. Both the extracts improved the activities of enzymic antioxidants (SOD, CAT, GPx and GST) and the levels of non-enzymic antioxidants (vitamin C, E and A, and GSH) with differential antioxidant response, resulting in the decreased levels of lipid peroxidative products such as conjugated dienes (CD), hydroperoxides (HP) and malondialdehyde (MDA) in liver. BBL was found to evoke a better antioxidant activity compared to RBL. The results observed with the antioxidant and biochemical parameters in the experimental animals were also confirmed with the histological architecture of the liver.

Histopathological results showed that the ethanol-CCl_4 treatment induced centrilobular necrosis and fatty changes of the hepatocytes, and sparing of hepatocytes around the portal tract. These changes were found to be reduced tremendously in BBL and RBL treated, oxidatively stressed animals. The liver of the animals treated with BBL and RBL showed normal histological picture indicating their non-toxicity.

The anticancer effect was evaluated in DLA tumour induced mice. The DLA tumour induced mice showed a high mortality rate and increase in the body weight with significant changes in the hematological parameters (Hb, RBCs and WBCs) compared to the control group. The increase in survival time and decrease in the body weight indicated the anticancer effect of BBL and RBL extracts. Modification in the hematological parameters indicates their preventive effect of BBL and RBL extracts. Comparatively BBL was found to exhibit a better anticancer effect than the RBL.

The overall analysis of the results obtained in the first three phases of this study definitely gave indication of a difference in the biochemical mechanisms by which the leaves of the plants of the two varieties (i.e., BBL and RBL) exerted their antioxidant activities. The leaves of black berried plants (BBL) improved the antioxidant components of all categories, in both enzymic and non-enzymic. The

leaves of red berried plants (RBL) also improved enzymic and non-enzymic antioxidants but to a lower extent than BBL in most components.

The interesting trend noted was that glutathione-related components responded better to RBL exposure than BBL, suggesting that RBL exerts its action via a glutathione-dependent mechanism, while BBL adopts a different, as yet unidentified mechanism. This trend was observed both *in vitro* and *in vivo* lending strength to the above suggestion.

However, the overall response of the leaf extracts on biomolecules (lipids and DNA), intact cells, tissue slices (antioxidant status and LPO), and in animals (antioxidant status, LPO and tumour burdon) shows that BBL is a better protector than RBL. Thus, it can be deduced that the antioxidant response evoked via the proposed glutathione-dependent mechanism is less efficient than the other. More in-depth study to understand the actual components involved in the protective mechanism of the leaves are needed to pinpoint the actual players in this process.

In the fourth phase, nature of the active principles in BBL and RBL were analysed. Preliminary phytochemical analysis revealed the presence of phenols, flavonoids and alkaloids. TLC also indicated the presence of phenols and alkaloids.

To confirm the nature of the active components present, the spectral analysis (FT-IR, NMR and GC-MS) were conducted. These analyses revealed the possibility of the presence of the flavonoidal and terpenoidal or steroidal glycosides.

The present investigation, thus, confirms the antioxidant and anticancer effects of two varieties of *Solanum nigrum* leaves i.e. BBL and RBL. All these results lend justification to the reputation of the leaves of these plants in the preparation of medicine for preventing and treating oxidative stress-associated diseased conditions including cancer, liver diseases, CVD, neurological disorders, diabetes, skin diseases, and age-associated disorders.

SUGGESTIONS FOR FUTURE RESEARCH

The outcome of the present study has opened up several promising avenues of possible research. Some of them that can be followed up for active research are as follows :

- The components present in the leaves can be fractionated into different solvent extracts and used to study their antioxidant and anticancer effects in comparison with the crude aqueous extract.

- The antioxidant and anticancer effects of isolated active principles can be studied.

- Varying doses of the leaf extracts can be analysed to optimize the maximally beneficial dose.

- A comparative analysis of the antioxidant potential of the other parts of the plant can be taken up (like raw berries, ripe berries, flowers, stem and roots).

- The anticancer potential can be further probed using cancer cells lines as well as other cancer models.

- The effect of the leaf extracts can be studied on stress induced by toxins and oxidants other than CCl_4 and H_2O_2.

- The effect of the cooking process on the antioxidant property can be evaluated, as *Solanum nigrum* leaves are used as a leafy vegetable.