



*Summary and Conclusion*

## 6. SUMMARY AND CONCLUSION

Herbal plants form a major part of traditional medicinal systems like Ayurveda, Siddha and Unani. The importance of medicinal plants is now being recognized even by the modern medicine. Natural products, due to their easy availability, low cost and lack of side effects, make themselves attractive candidates for drug research.

Ayurveda is a traditional and most commonly practiced form of medicine in India. Ayurvedic herbs stimulate the function of specific organs in the body, possibly by altering hormones affecting immunity and neurotransmission, and conveying antioxidant properties. The use of various parts of several plant drugs as indigenous medicine to cure specific ailments has been in vogue from ancient times.

Many of today's drugs are derived from plant sources. Plants have been used in medicine throughout the world and still continue to occupy an important place in traditional as well as modern system of medicine. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties.

One such medicinal plant is *Triticum aestivum* (wheat grass) which has been called as one of the nature's finest medicines and known for its nutritive value. The present study was formulated to understand the antioxidant properties of the *Triticum aestivum* leaves.

In the first phase of the study, the levels and activities of non-enzymic and enzymic antioxidants in the leaves of *Triticum aestivum* were assessed. The leaves were studied at three different time points of their growth namely 4, 8 and 12 days after sowing in order to determine any change in their antioxidant content in different stages of its growth. From the results obtained, the leaves on the 4<sup>th</sup> day

of growth were found to possess maximum antioxidant content followed closely by the 8<sup>th</sup> and 12<sup>th</sup> day of growth.

In the second phase of the study, the *Triticum aestivum* leaf extracts were tested for their radical scavenging effects and biomolecular protective effects under oxidatively stressed conditions on purified biomolecules, isolated cells and *in vitro* systems which simulated the *in vivo* environment.

To identify the ideal solvent into which maximum amount of antioxidants got extracted into and the nature of the active components, the leaves were serially extracted into the solvents of differing polarity (petroleum ether, benzene, ethyl acetate, methanol and water). A fresh juice (crude homogenate in water) was also prepared.

All the extracts were tested for their radical scavenging properties against superoxide and nitric oxide. Among all the extracts tested the fresh juice of the 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> day *Triticum aestivum* leaf extracts were very effective radical scavengers followed closely by the methanolic extract, implying that the active components contributing to the antioxidant activity are predominantly polar in nature. Hence the best radical scavenger, fresh juice (aqueous extract) was the choice of our study for further analysis.

The fresh aqueous extracts were then tested for their scavenging effect against DPPH and *in vitro* generation of hydroxyl radicals and hydrogen peroxide. The fresh juice of the 4<sup>th</sup> day extract was found to be a very effective scavenger, followed closely by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts. Thus, the *Triticum aestivum* leaf extracts possessed both considerable level of antioxidants and radical scavenging properties.

Oxidative stress results in an excess of free radicals, which can react with cellular lipids, proteins and nucleic acids, leading to local injury and eventual

organ dysfunction. Lipids are probably the most susceptible biomolecules to free radical attack followed by DNA molecules. Therefore, to evaluate the mechanism by which the *Triticum aestivum* leaves elicit antioxidant properties, its effect on lipid and DNA preparations were studied.

The effect of the *Triticum aestivum* leaf extracts were analyzed on three different membrane lipid preparations namely plasma membrane, mixture of plasma and internal membrane and intact cells on exposing to oxidant stress *in vitro*.

It was observed that a better protection was rendered by leaf extracts to the mixture of plasma membrane and internal membrane which implies that an endogenous factor may act along with the plant components to elicit its antioxidant properties.

Oxidizing radicals readily attack DNA if they are formed in its vicinity and is therefore a vulnerable and important target. Hence the potentiality of the *Triticum aestivum* leaf extracts were analyzed for its protective effects against purified DNA preparations, as well as the DNA in intact live cells.

The protection rendered by the *Triticum aestivum* leaves upon oxidative damage to DNA was followed in commercially available DNA prepared from sources of varying hierarchies of evolution. They were  $\lambda$  DNA (linear, viral), pUC18 (plasmid, bacterial) and herring sperm (haploid, eukaryotic) DNA. The results revealed that the damage was not completely reverted back in the  $\lambda$  DNA and damage was still present in the case of pUC18 DNA whereas the reversal was complete in the DNA of the intact cells.

The *Triticum aestivum* leaf extracts proved to be very efficient in improving the survival of cells upon toxic oxidant assault, which suggests its candidature as a support to anticancer therapy.

The results showed that the *Triticum aestivum* leaf extracts were very effective in protecting the primary targets of oxidative assault namely lipids and DNA against oxidant induced damage.

Further to this, the *Triticum aestivum* leaf extracts were analyzed for their antioxidant activity *in vitro* in precision-cut goat liver slices against H<sub>2</sub>O<sub>2</sub> induced toxicity. Enzymic and non-enzymic antioxidants were analyzed in the liver slices subjected to oxidative stress, in the presence and the absence of the leaf extracts. The enzymic antioxidants analyzed were SOD, CAT, POD, GR and GST and the non-enzymic antioxidants assessed were ascorbate,  $\alpha$ -tocopherol, vitamin A and GSH.

H<sub>2</sub>O<sub>2</sub> induced damage to the liver slices was reflected by the decreased antioxidant enzyme activities and the levels of non-enzymic antioxidants when compared to controls. Co-treatment of the liver slices with *Triticum aestivum* leaf extracts prevented these changes from occurring on exposure to H<sub>2</sub>O<sub>2</sub>. All the three extracts tested were capable of improving the levels of antioxidants studied to a significant extent. The 4<sup>th</sup> day leaf extract was most potent in this regard, followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

The results obtained in this phase proved that the *Triticum aestivum* leaf extracts possessed strong antioxidant efficacy in the *in vitro* systems studied.

The third phase of the study involved the confirmation of the results obtained under *in vitro* conditions using experimental rats. In this phase, male Wistar rats were subjected to oxidative challenge by alcohol and the oxidant CCl<sub>4</sub> and the ability of the fresh aqueous leaf extracts of *Triticum aestivum* to counteract the damage was analyzed.

The circulating levels of the liver marker enzymes and the lipid profile analyzed indicated that the ethanol-CCl<sub>4</sub> imposed a strong oxidative stress, which

was effectively mitigated by the *Triticum aestivum* leaf extracts to a significant extent.

The antioxidant status of the animals was analyzed by assessing the enzymic and non-enzymic antioxidants in the liver (metabolic organ) and the kidney (excretory organ) tissues. The end product of oxidative damage namely lipid peroxidation and the DPPH scavenging ability of the liver tissues were also investigated.

The antioxidant activities were found to be drastically decreased by the ethanol-CCl<sub>4</sub> administration in both the liver and kidney tissues, whereas the antioxidants were effectively restored by the fresh aqueous extracts of *Triticum aestivum* leaves. The leaf extracts, by themselves, boosted the antioxidant status in both the liver and kidney. However, a differential response was observed in the liver and kidney tissues upon exposure to oxidant stress and also in the presence of *Triticum aestivum* leaf extracts.

The *Triticum aestivum* leaf extracts also protected the liver tissues from oxidant induced lipid peroxidation and also possessed better DPPH radical scavenging ability. The effects elicited by the *Triticum aestivum* leaf extracts were comparable to that of the standard antioxidant, silymarin. This phase of the study further proved the antioxidant potential of *Triticum aestivum* leaves with the maximum activity shown by the 4<sup>th</sup> day leaf extract followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

The ameliorating potential of *Triticum aestivum* leaf extracts against ethanol-CCl<sub>4</sub> induced toxicity was also confirmed by the histological findings in the liver and kidney tissues.

In the fourth and final phase of the study, an attempt was made to identify the active component in the leaf extracts of *Triticum aestivum*. Preliminary

phytochemical screening of the leaves identified alkaloids as the major antioxidant component in the leaves of *Triticum aestivum*.

To confirm the results obtained in the phytochemical screening, spectral analyses (FT-IR, <sup>3</sup>HNMR, <sup>13</sup>CNMR and GC-MS) were carried out. The spectral analysis showed the presence of steroidal alkaloids namely β-sitosterol and campesterol as major components.

Thus, the present study has confirmed the antioxidant potential of the *Triticum aestivum* leaf extracts under conditions of oxidative stress, both *in vitro* and *in vivo*. This observation strongly suggests that the *Triticum aestivum* leaves can be used in medicinal preparations to combat the disorders caused by oxidative stress.

#### **SUGGESTIONS FOR FUTURE RESEARCH**

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

- ◆ The anticancer activity of the *Triticum aestivum* leaf extracts can be probed using various cancer cell lines.
- ◆ The antioxidant activity of the *Triticum aestivum* leaf extracts can be further studied *in vitro* systems using other tissues like kidney.
- ◆ The effect of *Triticum aestivum* leaf extracts can be investigated against oxidative stress caused by different types of oxidants, acting by different mechanisms.
- ◆ The mechanism of the leaf extracts in protecting biomolecules can be probed further.
- ◆ The active components can be isolated and purified, and their effect can be studied further against oxidative stress.