CHAPTER – 7:

CONCLUSIONS

7. CONCLUSIONS

In the present study, an attempt was made to elucidate the immunopathological, haematological, biochemical, antifertility and neuromodulatory activities of crude (unboiled) and cooked (boiled) *Diplazium esculentum* (DE) by investigating several *in vivo* and *in vitro* parameters.

Both unboiled and boiled DE fed mice exhibit poor growth, decreased body weight and relative organ weight. Hematological as well as immunological studies showed significant alternations in blood parameters, such as total and differential leukocyte count, number of plaque forming cells, HA titre values, macrophage number, etc. in both unboiled and boiled DE fed mice. Decreased serum levels of Th1 and Th2 cytokines have been observed in both crude and boiled DE fed mice, whereas mouse lymphocytes incubated *in vitro* with methanolic extract of DE showed significant decrease in cell numbers as well as Th1 and Th2 cytokine production.

Subchronic and chronic doses of crude and boiled DE have been found to decrease the relative testis & other accessory organs weight, decrease the sperm count, sperm viability & motility, fertility index and fecundity, total protein in serum, testis & ovary, decrease the fructose and α-glucosidase level, decrease the cholesterol in testis, etc. Interestingly, the Th1/Th2 cytokine index has been increased significantly in some of the pregnant mice that were treated with both crude and boiled DE, i.e., Th1 cytokine expression is higher than Th2 cytokine in these mice that ultimately causes infertility and recurrent spontaneous abortion (RSA).

Methanolic extract of DE has been shown to possess acetylcholinesterase inhibitory activity. Acetylcholinesterase inhibitors on the other hand can cause a significant modulation of immunity as a side effect. Therefore, DE seems to modulate the cholinergic anti-inflammatory pathways via the protection of ACh from splitting by cholinesterases and thus enhancing the pathway and decline the production of different proinflammatory cytokines like IL-2 and IFN-γ. Methanolic extract of DE has been shown to possess a moderate quantity of flavonoids and phenolic compounds, which may confer the antioxidant and free radical scavenging activities in this plant. But boiled preparation of DE has been found to have trace amount of antioxidant and free radical scavenging activities.

D. esculentum has been found to possess several phytochemicals such as flavonoids, tannins, phenolic compounds, saponins, glycosides, terpenoids, etc. Some of these phytochemicals are beneficial, whereas, some are detrimental in nature. We have observed that both crude and boiled DE possess hemolytic activity. Saponins have the capacity to destroy cell membrane, therefore may be related to the hemolytic potential. On the other hand, tannins inhibit protein availability through denaturation. Tannins are heat resistant compounds that can withstand high temperature during boiling. Thus, the toxic effects observed in our study could be related to tannins and other heat stable compounds.

Serum biochemistry revealed increased level of several enzymes and metabolic products of liver and kidney in to the blood of both unboiled and boiled DE fed mice, indicating malfunctioning of these systems that may induces several metabolic diseases and age-related degenerative disorders which are closely associated with the oxidative processes in the body. Histopathologically, disorganized vasculature and other associated changes were seen in liver and kidney, in both crude and boiled DE fed mice. However, only crude DE fed mice showed progressive specific lesions, whereas, in case of boiled DE fed mice, distinct disorganization has been observed only in those mice that are treated with chronic doses (180 days) of boiled DE.

Therefore, it can be concluded that crude as well as boiled DE possess several phytochemicals, some of which is detrimental to health. The phytochemicals like steroids, tannins or saponins may interfere with the cell metabolism and therefore may be considered as toxic upon consumption. This may be a good reason of avoidance of this fern among insects and cattle. It is also important to note that, even after boiling, phytochemicals like tannins and saponins were present in DE, indicating its heat tolerance property, and therefore, people who consume it in a regular fashion, should be aware about the hazards of its consumption.