CHAPTER - 5
SUMMARY
&
CONCLUSION
Indian Ayurveda, Charak Samhita, Shrushuta and various other philosophical literatures reveal that plants and its products are potent and safe remedy for severe human diseases from ancient times. Several studies have also proved that plants contain many medicinal properties that may be used to cure many nutritional disorders and diseases in human health care system. Since, in last few decades there are some diseases like cancer, diabetes, cardio-vascular disease etc. are rapidly increasing and becoming a serious threat to mankind health across the whole world. Diabetes mellitus, the second most leading disease and major cause of human death, have special attention to cure it with most reliable, potent, and safe medicines. There are so many artificial chemical drugs that have rapid and effective response to clinical disturbances in diabetes but they are costly and having side effects. Complementary and alternative herbal pharmacological approaches have attracted enormous interest to cure this disease due to greater efficiency, free of side effect, and lower cost; at place of existing synthetic hypoglycaemic agents. In this prospect, searching of new phytomedicines along with synthetic medicine is continuing on time to time.

The present study was designed to screen the plants for in vitro total antioxidant activity and phytochemical strength; in vivo antioxidative and antidiabetic potentials of ethanolic extracts of three selected herbal plants viz. Oxalis corniculata of Oxalidaceae family; Phyllanthus fraternus of Euphorbiaceae family and different parts (fruits, leaves, stems and roots) of Trichosanthes cucumerina of Cucurbitaceae family using alloxan induced type-2 diabetic rats. Ethanolic extracts of plants and their parts were prepared by the standard method. A comparative studies of all these plants extract were also done with a commercial standard synthetic drug i.e. Glimiprex. The toxicity studies of ethanolic extracts of all selected plants were done before going to animal trial for scrutinizing antidiabetic activities. For animal trial, Charles Foster strain albino rats of both sexes (wt. 200 ± 20 gm) were used and animal study was ethically approved by IAEC of Mahavir Cancer Sansthan, Patna, Bihar. The experimental rats were grouped as mentioned below and were administered with plants extract at the dose of 200 and 400mg/kg BW (on the basis of their LD$_{50}$ values) to study their anti-diabetic & antioxidants activities. Histological studies of liver, kidney & pancreas of the experimental rats were also done.

- Group I- normal controlled healthy rats
- Group II- diabetic controlled rats
- Group III- diabetic rats treated with plants extracts

Group IV- diabetic rats treated with commercial drug Glimiprex as standard at a dose of 100 mg/kg BW.
Group III was divided further into eight sub groups to treat the rats with four different plants extract at the dose of 200 & 400 mg/kg BW. Each group contained 4 rats. Normal control rats and diabetic control rats had no treatments.

**In vitro total antioxidant activity and phytochemical screening of plants extract**

*In vitro* total antioxidant activity of ethanolic extracts of selected plants were evaluated by DPPH free radical scavenging method and outcomes presented strong scavenging activity of *P. fraternus* extract with IC$_{50}$ = 202.22 ± 1.89 µg/ml among all extracts. Fruits and leaves extracts of *T. cucumerina* exhibited higher scavenging activity (with IC$_{50}$ values 260.30 ± 2.23 µg/ml and 278.29 ± 2.98 µg/ml respectively) than stem and root extracts. Thus, ethanolic extracts of *P. fraternus*, *O. corniculata*, fruits and leaves of *T. cucumerina* possessed good *in vitro* antioxidant activity.

In phytochemical screening of all plants extracts, the content of Total Phenols, Total Flavonoids, Total Carotenoids, Ascorbic Acid and Non Protein Thiol were estimated by using standard methods. Results exhibited that all extracts had good quantities of these phytocompounds. Ethanolic extract of *P. fraternus* displayed greater phytochemicals strength (2.14±0.12 mg total phenols, 19.67±0.68 mg flavonoids, 4.63±0.14 mg carotenoids, 1.39±0.16 mg ascorbic acid and 33.50±1.14 µmole non protein thiols in 100mg RW) amongst other extracts whereas in different parts of *T. cucumerina*, fruits and leaves parts had higher phytochemicals than other parts. Furthermore, all ethanolic extracts had very less amount of total sugar that could not affect the sugar level of diabetic animal.

**LD$_{50}$ values and toxicity screening of plants extract**

Screening of oral acute toxicity of ethanolic extracts of *O. corniculata*, *P. fraternus*, fruits and leaves of *T. cucumerina* in rat models indicated that there was no mortality found at the dose of 200 mg/kg BW/day and 400 mg/kg BW/day, while 100 % mortality was seen at the dose of 2000 mg/kg/day BW within 72 hours of dose administration. The LD$_{50}$ values were found as 1300, 1125, 1400 and 1300 mg/kg BW (oral) of ethanolic extracts of *O. corniculata*, *P. fraternus*, fruits and leaves of *T. cucumerina* respectively. Thus use of plants extracts as therapeutically would be safe below their LD$_{50}$ values. Again, two doses i.e. 200 and 400 mg/kg BW were monitored for behavioral and physiological toxicity signs (like inappetence, depression, aggressiveness, respiratory distress, body weight loss and death) for 7 days and results showed no toxicity signs. Results of hepatotoxicity and renotoxicity of the extracts showed no significant change in level of hepatorenal functions markers and cellular architecture of liver and kidney.
In vivo Antidiabetic and antioxidative studies of plants extract in alloxan induced DMT-2 rats

Physical observations (like Body weight, Skin colour, Skin hair, Body activity) in alloxan induced diabetic rats after treatment with extracts at the dose of 200 and 400 mg/kg BW imparted improvements in changes of physical parameters in comparison to diabetic control. At the dose of 400 mg/kg BW, all ethanolic extracts treated diabetic rats showed significant increase in their body weight and got back their white skin colour, normal skin hair, normal body activity from yellowish skin colour, dull & yellowish skin hair, moderate body activity of diabetic control rats.

In the study, the weight of vital organs such as liver kidney pancreas and spleen were found decreased by 10.69%, 19.27%, 18.75% and 21.42% respectively in diabetic rats than normal control rats. Further treatment with extracts of O. corniculata, P. fraternus, fruits and leave of T. cucumerina normalized their weights. Plants extracts treatments were found more effective in increasing the reduced weights of vital organs than standard drug Glimiprex, in diabetic rats.

Anti-Hyperglycemic property of plants extracts were studied in alloxan induced diabetic rats. Although, Glimiprex treatment showed highest reduction (60.57%) in fasting plasma glucose but plants extracts also had effective reductions at the dose of 400mg/kg BW when compared to diabetic rats. At the dose of 400mg/kg BW, P. fraternus extract treated diabetic rats displayed highest decrease i.e. 50.62% in fasting plasma glucose followed by O. corniculata extract (50.62%), T. cucumerina fruits extract (41.51%) and 39.42% in leaves extract.

Glycosylated hemoglobin (GHb) was also found increase with increase in plasma glucose level. Results showed 55.96% increment in blood GHb% level of diabetic rats than normal control rats and maximum decrement (11.48%) by standard drug Glimiprex treatment. Plants extracts treatments revealed little decrement than standard drug Glimiprex treatment. At dose of 400 mg/kg BW, P. fraternus and O. corniculata extracts treated diabetic rats exposed maximum decrease (11.68% and 9.71% respectively) followed by fruits and leaves extract of T. cucumerina on compared to diabetic control.

Diabetic dyslipidemia is characterised by hypercholesterolemia, hypertriglyciedemia and reduced HDL-cholesterol. Present study exhibited that treatment with extracts of O.corniculata, P. fraternus and fruits of T. cucumerina had maximum and almost equal decrement (34.27%) in total cholesterol level, while leaves extract showed minimum decrement (28.78%) in comparison to diabetic control. Similarly, fruits extract of T. cucumerina had maximum reduction (43.71%) followed by P. fraternus (37.45%), O. corniculata extract (33.28%) and least in leaves extract
(29.11%) at the dose of 400mg/kg BW. Unlikely, leaves extract of *T. cucumerina* showed maximum increase (64.99 ± 4.30 mg/dl) in HDL-cholesterol followed by fruits of *T. cucumerina*, *O. corniculata* and *P. fraternus* extracts. Standard drug Glimiprex treatment was also found to regulate dyslipidemic condition but not efficiently as plants extracts treatments.

Functional abnormalities in liver of diabetic rats were exposed by increased activity of enzymatic biochemical markers (such as GOT, GPT and ALP enzymes) in both plasma and liver tissue homogenate. Structural abnormalities comprised degeneration of hepatocytes, reduction of nuclei; expansion of sinusoidal space with rupture of central vein. Although, Glimiprex treatment to these diabetic rats showed decrement in these enzymatic biochemical markers, but not brought improvement in structural changes. While, treatments with *O. corniculata*, *P. fraternus*, fruits and leaves of *T. cucumerina* illustrated more significant decrement in increased level of GOT, GPT and ALP activities in both, plasma and liver of diabetic rats at the dose of 400 mg/kg BW. Even the plants extracts caused better improvement in altered structure of diabetic liver than standard drug Glimiprex treatment.

Kidney functions markers like urea, uric acid and creatinine level were found increased in plasma of diabetic rats and subsequently found decreased maximally by treatment with extracts of fruit and leaves of *T. cucumerina* at the dose of 400 mg/kg BW. However, treatments with *O. corniculata* and *P. fraternus* also showed decrement in these elevated markers but lesser than extract of fruits and leaves of *T. cucumerina*. Standard drug Glimiprex treatment to diabetic rats was not found as much effective as plants treatment. Structural changes in kidney of diabetic control rats involved damage of glomeruli, Bowman’s capsules, DCT and PCT. The damaged cellular structure of diabetic kidney got improved significantly after treatments with fruits and leaves extract of *T. cucumerina*; and little improvement was also observes by *P. fraternus* and *O. corniculata* extracts.

Pancreas is the key organ which is affected roughly and seriously in diabetes. In present study, the alloxan induced type-2 diabetic rats showed selective damages of β-cells and islets of Langerhans. Thus they had very low insulin level and also diminished effects of insulin to glucose uptake transporters. This in turn increased production of α-amylase and lipase enzymes. Experimental results displayed maximum decrease as 35.99% in α-Amylase activity and 41.85% in Lipase activity among all extract treatments by supplement of fruits extract of *T. cucumerina* followed by leaves, *O. corniculata* and *P. fraternus* extract treatment at the dose of 400 mg/kg BW. Standard drug Glimiprex treatment exhibited a little decrement in both enzyme activities.
Similar trends of improvements were found in structural changes of diabetic pancreas after treatments with above extracts and standard drug.

*In vivo* antioxidant activities of ethanolic extracts of *O. corniculata; P. fraternus;* fruits and leaves of *T. cucumerina* during diabetes were studied by estimating activities of enzymatic antioxidative markers (CAT, SOD, GSH-POX, GST, GPOX) and level of non enzymatic antioxidative markers (GSH, LPO, Vitamin-A, Vitamin-C) in plasma and liver of herbal treated and control rats.

Enzymatic and non-enzymatic antioxidative markers were found significantly reduced except LPO which was found increased in both plasma and liver of alloxanized rats. Plasma CAT activity of diabetic rat was found increased by 147.07%, 119.08%, 86.0% and 50.89% on treatment with extracts of *O. corniculata, P. fraternus*, fruits and leaves of *T. cucumerina* at the dose of 400 mg/kg BW respectively. Similarly, liver CAT activity of diabetic rat was found increased by 98.04%, 98.19%, 64.86%, and 7.20% on treatment with *O. corniculata, P. fraternus*, fruits and leaves of *T. cucumerina* at the dose of 400 mg/kg BW respectively.

Among all ethanolic extracts at the dose of 400mg/kg BW, the feeding of *O. corniculata* extract recorded maximum increase in both plasma (30.23%) and liver (17.65%) SOD activity of diabetic rats whereas treatment with fruits extract of *T. cucumerina* displayed 2 folds greater increments in plasma SOD activity than leaves extract, but lower than *P. fraternus* treatment. Diabetic rats possessed nearly similar increments (around 17%) in liver SOD activity after treatments with *P. fraternus* and fruits extract of *T. cucumerina*, while least increase (12.99%) with its leaves.

GSH-POX activity in plasma of diabetic rat was maximally increased by supplementation of ethanolic extract of *P. fraternus* (29.47%) followed by *O. corniculata* (21.28%); fruits (18.07%) and leaves (5.54%) extracts at the dose of 400 mg/kg BW. Similarly, liver GSH-POX activity of diabetic rat was found increased maximally by treatment with fruits extract of *T. cucumerina*, followed by *P. fraternus; O. corniculata* and least by leaves of *T. cucumerina*.

Reduced GST activity in plasma of diabetic rat was found increased maximally by *P. fraternus* (75.0%) followed by fruits extract (66.25%), *O. corniculata* (63.75%) and leaves extract (43.75%) treatment at the dose of 400 mg/kg BW. Likewise, liver GST activity of diabetic rat was found increased by 23.41%, 13.92%, 18.98% and 10.75% on treatment with extracts of *O. corniculata, P. fraternus*, fruit and leaf of *T. cucumerina* at the dose of 400 mg/kg BW respectively. Concurrently, treatments of plants extracts to diabetic rats also displayed increase in GPOX activity in both plasma and liver of diabetic rat. The order in effective increments of
reduced GPOX activity was found by these extracts as *O. corniculata, P. fraternus*, fruits and leaves of *T. cucumerina*.

In non-enzymatic antioxidants, Reduced Glutathione (GSH) level in plasma of diabetic rat was recorded decreased by 41.06% and that increased maximally (102.43%) by treatment with fruits extract of *T. cucumerina* followed by *O. corniculata* (93.72%), *P. fraternus* (58.88%) and leaves extract (44.94%) at the dose of 400 mg/kg BW. Correspondingly, liver GSH level of diabetic rat was found decreased by 27.07% and increased by 24.02%, 21.60%, 41.99% and 18.44% on treatment with *O. corniculata, P. fraternus*, fruits and leaves of *T. cucumerina* at the dose of 400 mg/kg BW respectively.

Treatment with ethanolic extract of *P. fraternus* recorded maximum increase (175.20%) in reduced level of vitamin-A in plasma of diabetic rat, followed by *O. corniculata*, fruits and leaves of *T. cucumerina* with 158.67%, 140.49% and 90.08% increment respectively at the dose of 400 mg/kg BW. In the same way, liver vitamin-A level of diabetic rat was found increased by 161.76%, 150.32%, 133.33% and 121.89% on treatment with extracts of *P. fraternus*, *O. corniculata*, fruits and leaves of *T. cucumerina* respectively at the dose of 400 mg/kg BW.

Vitamin-C level in plasma of diabetic rat was found decreased by 36.52% and increased maximally (70.17%) by *P. fraternus* extract supplement followed by *O. corniculata* (64.10%), fruits (59.34%) and leaves of *T. cucumerina* with 38.33% at the dose of 400 mg/kg BW. Similarly, decreased vitamin-C in liver of diabetic rat was found increased by 29.97%, 51.14%, 34.57% and 15.02% on treatment with ethanolic extracts of *O. corniculata, P. fraternus*, fruits and leaves of *T. cucumerina* respectively at the dose of 400 mg/kg BW.

Increased LPO value of diabetic rat plasma was examined decreased by 22.17%, 22.17%, 17.10% and 7.39% on treatment with ethanolic extracts of *O. corniculata, P. fraternus*, fruits and leaves of *T. cucumerina* respectively at the dose of 400 mg/kg BW. Similarly, liver LPO value of diabetic rat was found decreased by 38.17%, 34.42%, 36.53% and 24.59% on treatment with extracts of *O. corniculata, P. fraternus*, fruits and leaves of *T. cucumerina* respectively at the dose of 400 mg/kg BW. Treatment of diabetic rats with standard drug Glimiprex showed a little improvement in altered antioxidants level, that confirm its lesser efficiency than plants extracts in enhancing antioxidative strength in diabetes type-2 condition.

It is concluded from present study that ethanolic extracts of *O. corniculata; P. fraternus;* fruits and leaves of *T. cucumerina* are good antihyperglycemic, antidyslipidemic, antioxidative herbals and help in normalizing liver, kidney and pancreas functions in non insulin dependent Diabetes mellitus. Overall, *P. fraternus* shows better antidiabetic and antioxidative properties amongst
studied extracts. However, *O. corniculata* possesses good antioxidative activity whereas fruits of *T. cucumerina* are good anti-diuretic. Concomitantly, all extracts at the dose of 400mg/kg BW reveal better antidyslipidemic, anti-diuretic and antioxidative activity than standard commercial synthetic drug Glimiprex. The study also confirms *in vitro* antioxidative activity (DPPH free radical scavenging activity) of these herbal extracts and presence of phytochemicals such as phenols, flavonoids, carotenoids, ascorbic acid and non protein thiols. Thus, ethanolic extracts of *P. fraternus; O. corniculata* and fruits of *T. cucumerina* may be used as pharmacotherapeutics with other phytomedicines for overcoming of type- 2 Diabetes mellitus complications. However, Exploration and establishment of bioactive compounds of extracts of studied plants and their mechanism to control diabetic disturbances as well as other diseases are still need to be further studied.