Chapter 6

DISCUSSION OF RESULTS
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**Chapter -6: Discussion of Results**

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Chapter 6
DISCUSSION OF RESULTS

6.1. Discussion on Results of Standardization of Polyherbal Formulation

According to WHO, determination of the macroscopic and microscopic study of the formulation is the primary step towards establishing its character and purity should be conceded before any tests are undertaken. In the current work the macroscopic study of formulation was carried out. The results of macroscopic study might be useful for distinctiveness from its substitutes and adulterants. There is no foreign organic matter was present in the formulation.

The physico-chemical parameters are supportive in assessment of the purity and quality of the drug. Loss on drying at 105°C for formulation was found to be 6.89%. It signifies the sizeable amount of moisture in formulation.

Ash values were used to notice the presence of any siliceous pollution and water soluble salts. These values are significant quantitative standards as they are useful in determining authenticity and clarity of drugs. The results suggest that, formulation have high water soluble extractive value than ethyl acetate, chloroform, methanol, petroleum ether soluble extractive values. The water soluble extractives state the presence of water soluble matters such as flavonoids, carbohydrates etc. The fluorescence nature of powdered
drug plays an elementary role in the determination of quality and purity of the drug substance. The results of preliminary phytochemical screening showed the presence of various phytoconstituents in the formulation, like carbohydrates, steroids, cardiac glycosides, flavonoids, alkaloids, tannins and phenolic compounds. The flow property of formulation was determined by Hausner’s ratio, Carr’s index, true density, bulk density $^{105,106}$. Formulation was tested pessimistic for the presence of heavy metals. For safe use of polyherbal formulation microbial content was checked whether total bacterial count, total yeast and mould count are within the approved limits and for the absence of micro-organisms like salmonella, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa$^{107}$. The outcomes which were obtained in this study may be measured as tools for support to the regulatory authorities, scientific organization and manufacturers for developing standard formulation.

6.2. Discussion on Results of Nephroprotective Effect Polyherbal Formulation

- Diabetes is now accepted as one of the major killer disease and a primary cause of death, claiming many lives world over. $^{108}$

- Diabetic nephropathy, a frequent and major microvascular complication of diabetes mellitus, is the most common cause of end-stage renal disease in many countries of the world. $^{109}$
• Several factors, such as hyperglycemia, Hyperlipidaemia, oxidative stress and inflammatory cytokines, contribute to the progression of renal damage in diabetic nephropathy\textsuperscript{110}.

• A huge number of hypoglycemic/antidiabetic plants and herbs are known in traditional medicine but their opening into contemporary therapy waits pharmacological testing by recent methods.

• The present results suggest that polyherbal formulation demonstrated significant antidiabetic, hypolipidemic and nephroprotective effects in STZ induced diabetic nephropathy.

• The polyherbal formulation seems to be safe upto 5g/kg because even at this high dose no toxic or deleterious effects were seen immediately or during 14 days of observation period.

• STZ-induced diabetes characterized by a severe loss in body weight induced by the degradation of structural proteins was connected with characteristic symptoms of diabetes.\textsuperscript{111}

• Diabetic nephropathy rats treated with PHF the weight loss was found to be reversed.

• The treatment with PHF normalized the food and water intake.

• The capacity of PHF to protect body weight loss seems to be as a end result of its ability to protect from diabetes.
• The total kidney weight and kidney weight/body weight ratio of diabetic animals was found to be increased when compared with normal control animals.

• This may be due to bulge of lining cells of tubules, fatty infiltration, large area of haemorrhage and lymphocyte infiltration in STZ-induced diabetic rats.\textsuperscript{112}

• In this study oral administration of PHF at doses 250, 500mg/kg b.w. notably decreased the kidney weight and kidney weight/body weight ratio in a dose dependent manner.

• Fasting blood glucose level in diabetic rats is a vital basal parameter for monitoring diabetes.\textsuperscript{113}

• In this study oral administration of PHF at doses 250, 500mg/kg b.w. significantly decreased the blood glucose level in a dose dependent manner.

• This effect on blood glucose is reflected in the glycosylated haemoglobin level.

• In the present study, diabetes was induced by STZ. STZ produces hyperglycemia by selective cytotoxic effects on pancreatic \( \beta \) cells. One of the intracellular phenomenon for its cytotoxicity is through production of free radicals.\textsuperscript{114}

• It has been resulted in decreased insulin production.
• In this study oral administration of PHF at dose 250, 500mg/kg b.w. significantly increased the insulin production in a dose dependent manner.

• Increased plasma insulin levels in the treated animals due to the regeneration or the protection of pancreatic β-cells.\textsuperscript{115}

• Glycogenesis in liver was mainly regulated by insulin. The decreased levels of liver glycogen were observed in diabetic nephropathy rats. This may be due to the diminutive levels of insulin in diabetic nephropathy state or oxidative stress by diabetes may inactivate the glycogen synthase.\textsuperscript{116}

• In this study oral administration of PHF at doses 250, 500mg/kg b.w. significantly increased the liver glycogen content in a dose dependent manner.

• The probable ways of antidiabetic action of PHF may be by inhibiting the inactivation of the glycogen synthetase and by synthesizing the glycogen synthetase.

• Diabetes mellitus patients frequently associated with dislipidaemia and are measured as a hazard factor for the development of diabetic nephropathy.

• Hyperglycemia and Hyperlipidaemia act synergistically to induce renal damage.\textsuperscript{117}

• Increase in triglycerides, total cholesterol, LDL, VLDL, Atherogenic index and decrease in HDL levels in STZ-induced
diabetic nephropathy rats. This may be due to either increased breakdown of lipids or recruitment of free fatty acids from the peripheral tissues\textsuperscript{118}.

- Oral administration of PHF at doses 250, 500mg/kg b.w. significantly decreased the triglycerides, total cholesterol, LDL, VLDL, Atherogenic index and increased the HDL levels in a dose dependent manner.

- It clearly demonstrated the presence of hypolipidemic agents in the polyherbal formulation.

- Oral administration of PHF at dose 250,500mg/kg b.w. significantly recovered the RBC, PCV, Hb and decreased the WBC in a dose dependent manner.\textsuperscript{119}

- Uncontrolled hyperglycemia can produce reactive oxygen species (ROS) which is major risk for the complications of diabetes.\textsuperscript{120,121}

- Hyperglycemia causes an elevated production of advanced glycation end products (AGES) which could make possible free radical production to interrupt cellular function of the kidney that associates with turn down in the endogenous ROS scavengers.\textsuperscript{122}

- Reduction in antioxidants like SOD, Catalase, Glutathione peroxidase, GSH and increased levels of MDA were observed in STZ-induced diabetic rats.
• Oral administration of PHF at doses 250, 500mg/kg b.w. significantly restored the SOD, GSH, Glutathione peroxidase, Catalase and decreased the lipid peroxidation in a dose dependent manner.

• Diabetic nephropathy characterized by elevated levels of serum creatinine, blood urea nitrogen, urinary albumin excretion rate, creatinine clearance.

  * Albuminuria is a symbol of DN and urinary albumin excretion rate is also certificated to be the best clinical predictor of renal lesions in DN.\textsuperscript{123}

• Creatinine is breakdown product of creatinine phosphate in muscle and its clearance is measured is an sign of GFR.

• UAER, serum creatinine, blood urea nitrogen, were higher in STZ-induced diabetic rats and decreased urine volume, GFR was observed.

• Oral administration of PHF at doses 250, 500mg/kg b.w. significantly decreased the levels of UAER, serum creatinine, blood urea nitrogen and significantly increased the urine volume, urinary urea, GFR in a dose dependent manner.

• Proteinuria is measured as a main sign of diabetic nephropathy. Little amount of proteins come out in the urine as a result of normal development of cell turnover and metabolism. Their
release is enhanced throughout the kidney’s functional impairment as happens in diabetes.¹²⁴

- In the present study proteins appeared in the urine of STZ-induced diabetic nephropathy rats. Higher amounts of protein in the urine indicate the development of DN.

- Oral administration of PHF at doses 250, 500mg/kg b.w. significantly decreased the levels of urinary protein excretion that shows the development of renal functions in a dose dependent manner.

- The deposition of extracellular matrix in the glomerular mesangium and tubulointerstitium is a characteristic pathologic change in DN.¹²⁵

- Type IV collagen increased during diabetes, was excreted in higher amounts in STZ-induced diabetic rats. It suggests that increased degradation is a result of diabetic condition.

- Oral administration of PHF at doses 250, 500mg/kg bw significantly decreased the excretion of type IV collagen in a dose dependent manner.

- Renal enlargement was marked by decreased amount of GAGS in kidney and increased urinary excretion. This could be due to decreased synthesis or increased degradation.¹²⁶

- Decreased levels of GAGS and increased excretion of GAGS were observed in diabetic rats.
• Oral administration of PHF at doses 250, 500mg/kg b.w. significantly increased the GAGS and decreased the excretion of GAGS in a dose dependent manner.

• Diabetic nephropathy has been demonstrated to be an inflammatory disease worldwide. Nowadays, accumulating evidences showed that hyperglycemia could mediate the alteration of extracellular and intracellular metabolism, such as the function of AGES.127

• The advanced glycation end products in kidney initiates pathological changes like hypertrophy and glomerular basement membrane thicknening, and increased aldosterone by angiotensin-II are responsible for increased Na⁺ reabsorption and increased potassium excretion128.

• An increased amount of AGES were observed in diabetic nephropathy kidney.

• Oral administration of PHF at dose 250, 500mg/kg b.w. significantly decreased the AGES, in a dose dependent mode.

• Clinical trials also showed that the improved accumulation of AGES in vascular tissues might induce the expression of proinflammatory cytokines.129

• IL-6 has been demonstrated to be a secondary inflammatory cytokine in disease risk that could be elevated in diabetic nephropathic condition.
• TGF-β1, one of the main subtypes of TGF-β, also has been demonstrated to be one of the key factors in modulating diabetes and its complications.

• TNF-α correlated with urinary albumin excretion and progression of DN.

• In current study increased levels of inflammatory mediators like IL-6, TGF-β1, TNF-α were observed in STZ-induced diabetic nephropathy rats.

• Oral administration of PHF at doses 250, 500mg/kg b.w. significantly decreased the levels of IL-6, TGF-β1, TNF-α in a dose dependent manner.

• The biochemical parameters were associated with the renal histopathological changes. In truth we discovered that STZ caused an important damage in renal structure showing noticeable glomeruli and tubular damages, haemorrhagic conditions. This was most likely due to the generation of reactive radicals and to succeeding lipid peroxidation induced by STZ.

• Oral administration of PHF at doses 250, 500mg/kg b.w. improves the histological alterations induced by STZ which could be accredited to its antiradical/antioxidant activities.
6.3. Discussion on results of Aminoguanidine against Diabetic Nephropathy.

Pharmacologic agents that specifically inhibit the process of nonenzymatic glycation are now under extensive investigation in terms of delaying the development and progression of diabetes-related complications. To date the most promising agent under investigation is aminoguanidine.

- In the present work, administration of AGE inhibitor, aminoguanidine during the induction of diabetes for 16 weeks ameliorated the renal dysfunction as assessed by reduced serum creatinine, urinary total protein, and urinary albuminuria irrespective of persistent hyperglycaemia.

- Our results are in harmony to those observed by other investigators.\textsuperscript{130, 131, 132}

- The ability of aminoguanidine to provide renoprotection indicates that its beneficial effects occur mainly by inhibiting AGE accumulation.

- Aminoguanidine contains a terminal amino group, which is of higher chemical reactivity than terminal amino groups of proteins. For this feature, aminoguanidine can inhibit glucose binding to proteins.

- Aminoguanidine induced a significant improvement in the deleterious effect of diabetes on lipid profile independent of
glucose control. It decreased serum levels of total cholesterol, triglycerides, LDL, VLDL, Atherogenic index.

- These lipid lowering effects of aminoguanidine could result from either primary effects of the drug on lipid metabolism or secondary effects related to AGE inhibition.

- Aminoguanidine induced a significant improvement in antioxidants like superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase, lipid peroxidation.

- Aminoguanididine significantly decreased the cytokines in serum like IL-6, TGF-β and TNF-α in Diabetic nephropathy rats.

- The ability of aminoguanidine to provide renoprotection against STZ-induced diabetes has been documented by histopathological studies.