5. DISCUSSION

This study was carried out to evaluate the antidiabetic, antioxidant, hypolipidemic and safety of different solvent extracts of *Murraya koenigii*. The study was divided into six experimental parts.

Experiment I was conducted to screen the antidiabetic activity of aqueous, petroleum ether, chloroform and methanol extracts of *Murraya koenigii* on oral administration in alloxan-induced diabetic wister rats for a period of 8 weeks.

Experiment II was conducted to elucidate the antidiabetic, antioxidant and hypolipidemic activity of aqueous and methanol extracts of *Murraya koenigii* on oral administration for a period of 12 weeks, which were chosen, based on the results of experiment I.

In the experiment III the freeze-dried aqueous extract of *Murraya koenigii* was evaluated for antidiabetic, antioxidant and hypolipidemic activity on intraperitoneal administration for a period of 4 weeks.

In the experiment IV oral glucose tolerance test was conducted using aqueous, methanol and freeze-dried aqueous extracts to elucidate the acute effect of these extracts on attenuating rise in blood glucose levels in Alloxan diabetic wister rats.

The experiment V was the continuation of experiment II, in this experiment the protein expression profile in pancreas of animals treated with aqueous and methanol extracts were analysed.

In the experiment VI, the 28 days repeated oral dose acute toxicity study was conducted to elucidate the safety of two promising extracts aqueous and methanol extracts as revealed by the experiment II. The results obtained by these studies are discussed individually below.
5.1 Experiment I

In the present study four solvent extracts of *Murraya koenigii* leaf were evaluated for antidiabetic activity in Alloxan induced diabetic rats. The petroleum ether, aqueous, ether and methanol solvent extracts of *Murraya koenigii* leaf were tested at the two doses 100 mg/kg b.w. and 1000 mg/kg body weight in order to cover the wide range of doses. Aqueous and methanol extract showed significant reduction in plasma glucose and increase in insulin levels at the dose of 1000 mg/kg body weight compared to concentration of diabetic control group on respective days.

At the end of the 8 weeks study aqueous extract significantly reduced (P<0.05) the plasma glucose levels to 179.8±21.5 mg/dl and methanol extract significantly reduced plasma glucose levels to 181.6±24.3 mg/dl as compared 270.4±20.9 mg/dl in control diabetic group. Petroleum ether and chloroform extracts did show significant reduction in plasma glucose levels at the same doses.

Aqueous extract significantly increased (P<0.01) the plasma insulin levels to 27.48±0.97 μU/ml and methanol extract significantly increased (P<0.01) the plasma insulin level to 28.48±0.76 μU/ml as compared to 21.96±0.76 μU/ml in control diabetic group. Petroleum ether and chloroform extracts did show significant increase in plasma insulin levels at the same doses.

*Murraya koenigii* leaves have been attributed with antidiabetic activity and many studies were conducted on *Murraya koenigii* have reported hypoglycemic and antidiabetic activity. However, most of the studies reported in the literature are about the whole leaf powder. There are very few studies with specific solvent extracts of *Murraya koenigii* leaves.

The decrease in the plasma glucose may be attributed to many bioactive phytochemicals present in the aqueous and methanol extract of *Murraya koenigii*. The
hypoglycemic activity of aqueous extract of the leaves of *Murraya koenigii* after oral as well as intravenous administration to normal and alloxan diabetic dogs reported by Narayana and Sastry (1975). Crushed leaves of *Murraya koenigii* reported to produce hypoglycemic activity in rabbits, human volunteers and alloxan induced diabetic rats (Santhakumari *et al.* 1987). Curry leaf powder supplementation (12 g providing 2.5 g fibre) to 30 non-insulin dependent diabetes mellitus patients for a period of 1 month resulted in the transient reduction in fasting and post-prandial blood sugar levels (Iyer and Mani, 1990). Methanol extract of *Murraya koenigii* leaves reported to produce hypoglycemia in human volunteers and alloxan induced rats and rabbits (Bhat, 1995: Rupashree, 1999). Feeding of diet containing various doses of curry leaf powder (5, 10 and 15%) to normal rats for 7 days as well as to mild and moderate diabetic rats for 5 weeks showed varying hypoglycemic and anti-hyperglycemic effect (Yadav *et al.*, 2002).

The hypoglycemic activity of *Murraya koenigii* was attributed to increased glycogenesis and decreased glycogenolysis and gluconeogenesis (Khan *et al.*, 1995) or due to induction of receptors and/or expression of gene/s required for insulin synthesis and release in the regenerated β-cells of pancreas (Bhat, 1995) or due to increased glucose uptake and its utilization by cells (Rupashree, 1999). In this study there was significant increase in the plasma insulin on administration of aqueous and methanol extract. Cold hexane extract of *Murraya koenigii* reported to have alpha amylase inhibitory activity and inhibits the conversion of carbohydrates in to simple sugars in the gut there by inhibits the entry of glucose in to circulation (Bawden *et al.*, 2002). *Murraya koenigii* supplemented diet showed to inhibit the development of insulin resistance and diabetes (Yadav *et al.*, 2004). Antidiabetic activity of methanol extracts of *Murraya koenigii* reported by Vinuthan *et al.*, (2005) in short term
experiment in diabetic rats. Hypoglycemic effect of single dose administration of aqueous extract of *Murraya koenigii* was reported by Kesari *et al.*, (2005). Recently Xie *et al.* (2006) reported the hypoglycemic and hypolipidemic activity of *Murraya koenigii* in ob/ob mice. Arulselvan *et al.*, (2006) and Narendhirakannan *et al.*, (2006) reported the antidiabetic affect of ethanol extract of *Murraya koenigii* in STZ induced diabetic rats. The results from present study confirm the earlier reported findings.

However in the present study petroleum ether and chloroform extracts did not show the either decrease in plasma glucose or increase in plasma insulin at the dose of 1000 mg/kg body weight. The probable reason for this finding may be absence of bioactive compounds in these extracts or the bioactive compounds may be present at too low concentration to exert their effect at the tested doses. This study clearly demonstrated the antidiabetic effect of Aqueous and methanol leaf extracts of *Murraya koenigii* in Alloxan induced diabetic rats.

**5.2 Experiment II**

The aqueous extract and methanol extracts were evaluated for antidiabetic, hypolipidemic and antioxidant property at dosage 200, 400 and 800 mg/kg b.w., for a period of 12 weeks. Glibenclamide 0.25mg/kg b.w. and Metformin 10mg/kg b.w. served as positive control. The results from this study are discussed in detail below.

**5.2.1 Antidiabetic activity**

Aqueous extract and Methanol extract of *Murraya koenigii* significantly reduced the plasma glucose levels and significantly increased the plasma insulin in 90 Days experiment in alloxan induced diabetic wistar albino rats.

Compared to concentrations of diabetic control group on respective Days Aqueous extract at 400 mg/kg b.w., showed a significant decrease in plasma glucose levels from Day 75 onwards and increase in plasma insulin from Day 60 onwards and 800 mg/kg b.w. showed a significant decrease in plasma glucose levels from Day 60 onwards and increase in plasma insulin from Day 30 onwards.
Methanol extract at 400 mg/kg b.w. showed a significant decrease in plasma glucose levels from Day 75 onwards and increase in plasma insulin levels from Day 60 onwards. At 800 mg/kg b.w. showed a significant decrease in plasma glucose levels from Day 60 onwards and increase in plasma insulin levels from Day 30 onwards.

The positive control compounds glibenclamide at 0.25 mg/kg b.w. showed significant decrease in plasma glucose levels from Day 45 onwards and significant increase in plasma insulin levels from Day 15 onwards. Metformin at 10 mg/kg b.w. showed significant decrease in plasma glucose levels from Day 75 onwards and did not show a significant change in the plasma insulin levels during 90 Days study. The present study showed the dose dependent antidiabetic activity of aqueous and methanol extract.

At the end of 90 Days, as compared to diabetic control group plasma glucose levels 276.21±19.07 mg/dl, the aqueous extract significantly reduced the plasma glucose to 186.29±20.08 mg/dl at 400 mg/kg b.w. (P<0.01) and to 164.39±19.34 mg/dl at 800 mg/kg b.w. (P<0.01). Methanol extract significantly reduced the plasma glucose to 184.61±17.51 mg/dl at 400 mg/kg b.w. (P<0.01) and to 160.07±16.27 mg/dl at 800 mg/kg b.w. (P<0.01). Glibenclamide at 0.25 mg/kg b.w. reduced the plasma glucose to 162.54±17.57 mg/dl (P<0.01) and metformin at 10 mg/kg b.w. reduced the plasma glucose to 181.37±18.71 mg/dl (P<0.01).

As compared to diabetic control group plasma insulin levels 21.08±0.91 μU/ml, aqueous extract significantly increased the plasma insulin to 29.31±1.71 μU/ml at 400 mg/kg b.w. (P<0.01) and to 36.74±1.42 μU/ml at 800 mg/kg b.w. (P<0.01). Methanol extract significantly increased the plasma insulin to 31.34±1.48 μU/ml at 400 mg/kg b.w. (P<0.01) and to 38.17±1.62 μU/ml at 800 mg/kg b.w.
Glibenclamide at 0.25 mg/kg b.w. increased the plasma insulin to 29.31±1.67 μU/ml (P<0.01) and metformin at 10 mg/kg b.w. did not significantly increase the plasma insulin.

Chemically Alloxan is 2-4-5-6-tetra-oxo-hexahydropyramidine. Alloxan induces oxidative free radical induced degeneration of pancreatic beta-islet cell to result in etiopathogenesis of diabetes mellitus resulting in hyperglycemia and reduced insulin (Chottopadhyay et al., 1997). Oral administration of aqueous and methanol extracts increased the plasma insulin levels and correspondingly decreased the plasma glucose levels in alloxan induced diabetic rats.

Narayana and Sastry (1975) reported the hypoglycemic activity of aqueous extract of the leaves of *Murraya koenigii* after oral as well as intravenous administration to normal and alloxan diabetic dogs. Recently Xie et al (2006) reported the hypoglycemic and hypolipidemic activity of *Murraya koenigii* in ob/ob mice.

The antidiabetic activity of *Murraya koenigii* leaf extracts can be attributed to multiple bioactive molecules present in the extracts. Phytochemical analysis the aqueous extract showed the presence of alkaloids, flavonoids, lactones, tannins and saponins. The methanol extract showed the presence of steroid, alkaloids, flavonoids, tannins, lactones, diterpenes and saponins.

Khan *et al.* (1995) reported the hypoglycemic action of *Murraya koenigii* was due to increased activity of glycogen synthetase in the liver, which increased glycogenesis or decreased activity of glycogen phosphorylase and gluconeogenic enzymes, which lead to decreased glycogenolysis and gluconeogenesis (Yadav, 2002; Kesari 2005).

Aqueous extract phytochemical constituents like Glutamic acid, glycine, histidine, isoleucine, ornitine, phenylalanine, serine, threonine and tryptophan reported to have glycogenic effect (West *et al.*, 1967). Other aqueous extract phytochemical constituents Alanine, arginine, leucine, lysine, nicotinic acid, calcium, iron, and vitamin C stimulate insulin secretion. Methanol soluble phytochemical
constituents like cadinene, dipentene, have hypoglycemic activity. (Tomassi et al., 1991; Radhakrishnan et al., 1955; Berne and Levy, 1988; Ananthasamy et al., 1996; Satyanarayana, 1999; Manjunatha et al., 2001).

We hypothesise bioactive molecules in aqueous and methanol extracts of *Murraya koenigii* might influence the cellular and molecular changes. The increased insulin levels may be due to enhanced regeneration and neogenesis of pancreatic beta cells, which was evident in the histology of rat pancreas from the groups, treated aqueous and methanol extracts of *Murraya koenigii* leaf extracts. Roberto et al., (2004) reported regeneration and neogenesis of pancreatic beta cells from either ductal or acinar cells in rats.

The other possible mechanism of antidiabetic activity of *Murraya koenigii* is due to increased glucose uptake and its utilization by cells by increasing GLUT2 expression and glucokinase activity. Alloxan concentration-dependently reduced the mRNA expression of Glucose transporter 2 (GLUT2) and Glucokinase (GK) and the effect on GLUT2 was more marked (Gai, 2004). Glucokinase is an important glucose sensor in pancreatic beta cell that mediates the production of insulin by pancreatic beta cells. GLUT2 helps in the glucose uptake and utilization from cells.

Ethanol extract of *Murraya koenigii* reported to increase insulin and C-peptide levels and glucose tolerance. Also induces the restoration of activities of carbohydrate-metabolising enzymes, such as hexokinase, glucose-6-phosphate dehydrogenase and glycogen synthase, in diabetic rats towards near normal (Khan, 1995; Yadav, 2004; Narendhirakannan et al., 2006)

The reference positive control drugs glibenclamide has significantly reduced the plasma glucose and elevated the plasma insulin levels. Glibenclamide belongs to
class of sulfonylureas. Sulfonylureas cause hypoglycemia by stimulating insulin release from pancreatic β-cells. Their effects in the treatment of diabetes, however, are more complex. Sulfonylureas also may further increase insulin levels by reducing hepatic clearance of the hormone. In the initial months of sulfonylurea treatment, fasting plasma insulin levels and insulin responses to oral glucose challenges are increased. With chronic administration, circulating insulin levels decline to those that existed before treatment (Krall, 1985). There has been controversy about whether or not sulfonylureas have clinically significant extrapancreatic effects (Beck-Nielsen, 1988). The concentration of insulin receptors increases in the monocytes, adipocytes, and erythrocytes of NIDDM patients who receive oral hypoglycemic agents (Olefsky and Reaven, 1976). Sulfonylureas enhance insulin action in cells in culture and stimulate the synthesis of glucose transporters (Jacobs et al., 1989). Sulfonylureas also have been shown to suppress hepatic gluconeogenesis (Blumenthal, 1977); however, it is not clear if this is a direct effect of the drug or a reflection of increased sensitivity to insulin.

Another reference drug metformin which belongs to biguanides group has shown significant decrease in plasma glucose but failed to increase plasma insulin. Metformin is antihyperglycemic, not hypoglycemic (Bailey, 1992). It does not cause insulin release from the pancreas and does not cause hypoglycemia, even in large doses. The main causes of reduced glucose levels during metformin therapy appear to be an increase in insulin action in peripheral tissues (Bailey, 1992) and reduced hepatic glucose output due to inhibition of gluconeogenesis (Stumvoll et al., 1995). Metformin also may decrease plasma glucose by reducing the absorption of glucose from the intestine.

Hence this study demonstrated the antidiabetic activity of Aqueous and methanol extracts of *Murraya koenigii*. Also it was concluded that the
Murraya koenigii extracts on multiple targets through its unknown mechanism of action.

### 5.2.2 Hypolipidemic activity

At the end of the 90 days study, aqueous and methanol extracts significantly decreased the levels of serum total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, phospholipids and total lipids and significantly increased HDL-cholesterol as compared to diabetic control group values 161.97±6.11 mg/dl, 95.12±4.91 mg/dl, 37.62±1.32 mg/dl, 188.14±5.81 mg/dl, 149.72±5.53 mg/dl, 499.83±17.68 mg/dl and 29.23±2.13 mg/dl respectively.

The respective levels in aqueous extract 400 mg/kg b.w treated group reduced to 111.54±6.28 mg/dl (P<0.05), 38.85±4.82 mg/dl (P<0.01), 30.87±1.07 mg/dl (P<0.01), 154.37±5.95 mg/dl (P<0.01), 120.15±5.28 mg/dl (P<0.01), 386.06±18.64 mg/dl (P<0.01) and increased to 39.19±3.16 mg/dl (P<0.05).

The respective levels in aqueous extract 800 mg/kg b.w treated group reduced to 90.09±6.27 mg/dl (P<0.01), 16.86±5.85 mg/dl (P<0.01), 28.67±0.92 mg/dl (P<0.01), 143.37±6.26 mg/dl (P<0.01), 112.42±5.43 mg/dl (P<0.01), 345.88±17.38 mg/dl (P<0.01) and increased to 41.82±3.24 mg/dl (P<0.05).

The respective levels in methanol extract 400 mg/kg b.w treated group reduced to 116.84±6.96 mg/dl (P<0.01), 43.89±4.93 mg/dl (P<0.01), 30.68±1.07 mg/dl (P<0.01), 153.41±5.95 mg/dl (P<0.01), 118.47±5.72 mg/dl (P<0.01), 388.72 mg/dl (P<0.01) and increased to 42.27±2.86 mg/dl (P<0.05).

The respective levels in methanol extract 800 mg/kg b.w treated group reduced to 89.86±6.73 mg/dl (P<0.01), 16.61±5.54 mg/dl (P<0.01), 27.83±1.02 mg/dl (P<0.01), 139.16±5.66 mg/dl (P<0.01), 107.59±5.76 mg/dl (P<0.01), 336.61±18.98 mg/dl (P<0.01) and increased to 45.42±3.13 mg/dl (P<0.01).
Metformin at 10 mg/kg b.w. treated group significantly (P<0.01) reduced total serum cholesterol to 136.18±5.17 mg/dl, LDL-cholesterol to 67.13±4.91, VLDL-cholesterol to 32.24±1.09 and did not significantly reduce the levels of triglycerides or phospholipids or total lipids or did not significantly increase HDL-cholesterol.

Glibenclamide at 0.25 mg/kg b.w. treated group did not significantly reduce the levels of serum total cholesterol or LDL-cholesterol or VLDL-cholesterol or triglycerides or phospholipids or total lipids or did not significantly increase HDL-cholesterol at the end of the 90 days study.

In an earlier study reports Murraya koenigii leaf powder in combination with Brassica juncea seeds reported to have decrease the levels of cholesterol and phospholipids. Rats supplemented with the addition of 10% curry leaf or 10% mustard seeds, at a level of 10% body weight for a period of 90 days resulted in a reduction in total serum cholesterol, LDL + VLDL, an increase in the HDL, lipoproteins and an increase in the LCAT (lecithin cholesterol acyl transferase) activity (Khan et al. 1996). Murraya koenigii supplementation to atherogenic diet was found to decrease plasma triglyceride, plasma phospholipid in male albino rats (Khan et al., 1998). Murraya koenigii extract was reported to produce hypolipidemic activity in ob/ob mice (Xie et al 2006).

Murraya koenigii contains tryptophan, vitamin C, lysine, arginine and niacin as water soluble constituents and niacin, vitamin E and β-carotene as methanol soluble constituents (Pruthi, 1979). Solubility of niacin in alcohol has been reported by West et al. (1967). Tryptophan is found to cause hypocholesterolemia through its effect on lipid metabolism indirectly as a manifestation of altered carbohydrate metabolism (Rogers and Pesti, 1992). Similarly, vitamin C, vitamin E and β-carotene are found to be hypocholesterolemic as they enhance bile acid synthesis (Manjunatha
et al., 2001) and inhibit cellular cholesterol biosynthesis (Fuhrman et al., 2000). Similarly, niacin is reported to be hypocholesterolemic (Satyanarayana, 1999).

Decrease in the serum lipids and triglycerides could be due to a low lysine:arginine ratio in plants. Vitamin C and niacin in aqueous extract and vitamin E and niacin in methanol extract of *Murraya koenigii* might have major role in decreasing serum lipids and triglycerides. Vitamin E, vitamin C and niacin have been ascribed to be hypotriglyceridemic (Sen and Mukherjee, 1997; Satyanarayana, 1999). Ingredients of the volatile oil of *Murraya koenigii* were found to have an hypotriglyceridemic effect (Bamosa et al., 2002).

Niacin has diverse actions affecting lipoprotein metabolism (Gey and Carlson, 1971; Brown et al., 1991; Drood et al., 1991). A primary effect appears to be decreased production of VLDL (Grundy et al., 1981), which may be due, at least in part, to a transient inhibitory effect of nicotinic acid on lipolysis, a decreased delivery of free fatty acids to the liver, and a decrease in triglyceride synthesis and VLDL-triglyceride transport. Enhanced clearance of VLDL also may occur, possibly owing to enhanced activity of lipoprotein lipase. The decrease in LDL levels could be due to decreased VLDL production and enhanced hepatic clearance of LDL precursors. Niacin also raises HDL cholesterol levels via mechanism(s) not yet understood, but a decrease in the clearance rate of apoA-I has been reported as well as a decreased synthesis of apoA-II has been reported (Blum et al., 1977; Shepherd et al., 1979).

Further, it was observed that plant proteins are hypocholesterolemic, hypotriglyceridemic and hypophospholipidemic principally due to a lower lysine:arginine ratio content (Rajamohan and Kurup, 1997). Aqueous and methanol extracts of *Murraya koenigii* have insulinotropic action. Insulin is found to cause an
increase in storage and decrease in release of lipids into circulation (Zammit, 1996). Insulin elicits a remarkable array of biological responses. The important target tissues for regulation of glucose homeostasis by insulin are liver, muscle, and fat, but insulin exerts potent regulatory effects on other cell types as well. Insulin is the primary hormone responsible for controlling the uptake, utilization, and storage of cellular nutrients. Insulin's anabolic actions include the stimulation of intracellular utilization and storage of glucose, amino acids, and fatty acids, while it inhibits catabolic processes, such as the breakdown of glycogen, fat, and protein (Granner, 1991).

Aqueous and methanol extracts of *Murraya koenigii* caused increase in the serum HDL-cholesterol. This is unique beneficial effect which prevents the deleterious effects of lipid peroxidation. When the cores of triglyceride-rich lipoproteins LDL-cholesterol, VLDL-cholesterol are hydrolyzed via lipoprotein lipase, there remain an excess of surface components, such as unesterified cholesterol, phospholipid, and various apolipoproteins. These materials are transferred to HDL, which act as a "sink" for them.

Hence, a decrease in the serum total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, phospholipids and total lipids concentration due to feeding of aqueous or methanol extract observed in this study could be due to presence of tryptophan, vitamin C, vitamin E, lower lysine:arginine ratio, niacin and β-carotene in *Murraya koenigii* extracts. Increase in HDL-cholesterol could be mainly attributed to niacin. The decrease in plasma total cholesterol could also be due to insulin secretogague activity of aqueous and methanol extract of *Murraya koenigii*. Compared to *Murraya koenigii* extracts glibenclamide and metformin did not show the hypolipidemic activity.
5.2.3 Antioxidant and Tissue protective activity

Aqueous and methanol extracts shown significant inhibition (P<0.01) of rise in serum creatinine, BUN, ALT, AST and bilirubin at all the tested doses 200 or 400 or 800 mg/kg b.w. At the doses 400 or 800 mg/kg b.w both aqueous and methanol extracts significantly elevated the activity of superoxidedismutase, catalase and significantly reduced the lipid peroxidation in liver, kidney, heart and pancreas.

Glibenclamide and metformin also ameliorated the raise in serum creatinine, BUN, ALT and AST. But they did not bring about any elevation in antioxidants superoxidedismutase or catalase activity or inhibit lipid peroxidation.

Elevated ROS levels have also been implicated in diabetes mellitus (Baynes, 1991; Wolff, 1993). Elevated glucose levels are associated with increased production of ROS by several different mechanisms (Oberley, 1988; Sakurai and Tsuchiya, 1988). Several independent strategies that ameliorate mitochondrial ROS production were shown to prevent some of the typical secondary complications of the diabetes including the formation of advanced glycation end products (Baynes, 1991).

In addition, superoxide is generated by the process of glucose auto-oxidation that is associated with the formation of glycated proteins (Van Dam et al., 1995). The interaction of advanced glycation end products with corresponding cell surface receptors stimulates ROS production and decreases intracellular antioxidants levels. The increase in ROS production contributes to the development of diabetic complications such as atherosclerosis and other vascular complications. In addition, hyperglycemia enhances cell-mediated low-density lipoprotein (LDL) peroxidation in endothelial cells. Treatment with antioxidants ameliorates diabetic complications (Ceriello et al., 1991).

In rats fed with *Murraya koenigii* leaf powder and *Brassica juncea* seeds there was a decrease in the concentration of malondialdehyde, while hydroperoxides and
conjugated dienes were increased in liver and heart. There was increased activity of Superoxide dismutase and catalase in liver and heart of administered groups. Glutathione levels in liver, heart and kidney were lowered in rats. Glutathione reductase, glutathione peroxidase and glutathione S-transferase activity showed a sharp increase in the administered group (Khan 1996).

According to Khan et al. (1997) addition of Murraya koenigii leaf powder in the high fat diet resulted in reduction of lipid peroxidation (thiobarbituric acid reactive substances) level to a beneficial extent. Histological studies also indicated the modulation of hepatic functions to near normal level. The present study findings correlate with the reported results.

Carbazole alkaloids isolated from Murraya koenigii are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases resulting from lipid peroxidation (Nakathani, 2000).

Oral feeding 15% of powdered leaves of Murraya koenigii and 10% powder of seeds of Brassica juncea for a period of 60 days to streptozotocin diabetic rats showed the nephro protective effect. There was improvement in serum glucose levels, body weight, urine volume, serum creatinine, and urinary albumin levels. Murraya koenigii could possibly be best utilized by promoting them as preferable food adjuvant for diabetic patients (Grover et al., 2003).

Baliga et al. (2003) reported the dose-dependent nitric oxide (NO) scavenging activity of aqueous leaf extract of Murraya koenigii. This study suggested that Murraya koenigii might be a potent and novel therapeutic agent for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite.

It was suggested that an aryl hydroxyl substituent on the carbazole rings play a role in stabilizing the thermal oxidation and rate of reaction against DPPH radical (Tachibana, 2003). Murraya koenigii treatment exerts a therapeutic protective nature
in diabetes by decreasing oxidative stress and pancreatic β-cell damage (Arulselvan and Subramanian, 2007).

Oxidative damage is widely considered to be a cause of diabetic complications (Marra et al., 2002; Penckofer et al., 2002). Recent proposals (Nishikawa et al., 2000) suggest that overproduction of reactive oxygen species (ROS) may be the initiating event leading to long-term development of diabetic complications. ROS, such as the superoxide radical, the hydroxyl radical, and hydrogen peroxide, are continuously produced in most cells under physiological conditions, and their levels are regulated by a number of enzymes and physiological antioxidants. Enzymes that detoxify ROS include superoxide dismutase (SOD; which produces hydrogen peroxide by dismutation of superoxide) and glutathione peroxidase, and catalase (the two enzymes that detoxify hydrogen peroxide). When the production of ROS exceeds the capacity of the cell to detoxify them, oxidative stress develops that is harmful to the integrity of biological tissue like pancreas, kidney, heart, liver, retina and neurons.

In the present study aqueous and methanol leaf extracts of Murraya koenigii showed improved histological appearance in major organs like liver, kidney, heart and pancreas. Particularly the rejuvenation and regeneration of pancreatic β-cell in alloxan induced diabetic rats was new feature observed in groups treated with aqueous or methanol extracts at the dose of 800 mg/kg b.w.

Carbazol alkaloids present in Murraya koenigii, euchrestine B, bismurrayafoline E, mahanine, mahanimbicine, and mahanimbine reported to have antioxidant activity (Tachibana, 2001). The vitamins C and E and niacin content present in Murraya koenigii extracts might have exerted antioxidant activity.

Decrease in the lipid peroxidation might be due to decrease in LDL cholesterol and thus reducing the oxidized LDL. Unlike native LDL, oxidated modified LDL
could recruit circulating monocytes, and then through uptake via the scavenger-receptor pathway, would have transformed them into foam cells. Endothelial and smooth muscle cell membrane injury by oxidated modified LDL has been postulated to play a role in atherosclerotic plaque development (Jose et al., 2005).

Hence aqueous and methanol extracts of *Murraya koenigii* seem to possess ameliorating effect on diabetes induced tissue damage in major organs like liver, kidney, heart and pancreas through the antioxidant and tissue protective activity.

### 5.3 EXPERIMENT- III

Freeze-dried aqueous extract of *Murraya koenigii* was evaluated for antidiabetic, antioxidant and hypolipidemic activity on intraperitoneal administration for a period of 4 weeks. This study revealed the extraordinary antidiabetic, antioxidant and hypolipidemic activity. In this study all the pharmacological activities observed at low dose of 25 or 50 mg/kg b.w. probably due to high level of bioactive molecules in the extract as the extract was prepared under controlled temperature and also may be due to route of administration. The discussion under 5.2.1, 5.2.2 and 5.2.3 in experiment II also hold good for this study.

#### 5.3.1 Antidiabetic activity

Intraperitoneal administration of different doses of freeze dried aqueous extract of *Murraya koenigii* showed a dose dependent antidiabetic activity. At the end of 4 weeks study freeze dried extract significantly reduced (P<0.01) the plasma glucose levels to 173.60±19.46 mg/dl at the dose 25 mg/kg b.w and to 161.91±21.72 mg/dl at the dose 50 mg/kg b.w. as compared to diabetic control group plasma glucose levels 276.16±17.83 mg/dl. Insulin levels significantly increased (P<0.01) to 28.43±1 µU/ml at the dose 25 mg/kg b.w. and 30.28±1.52 µU/ml at the dose 50 mg/kg b.w. as compared to diabetic control group insulin levels19.25±1.31 µU/ml.

#### 5.3.2 Hypolipidemic Activity
Intraperitoneal administration of different doses of freeze dried aqueous extract of *Murraya koenigii* showed a dose dependent hypolipidemic effect compared to diabetic control group parameters on respective Days.

Freeze dried aqueous extract at 25 or 50 mg/kg b.w showed hypolipidemic activity by significantly reducing the levels of serum total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, phospholipids and total lipids and significantly increased HDL-cholesterol at the end of the 28 days study as compared to diabetic control group values 103.72±6.37 mg/dl, 34.874±5.26 mg/dl, 28.38±1.41 mg/dl, 141.93±5.75 mg/dl, 108.46±4.75 mg/dl, 354.11±16.87 mg/dl and 40.46±2.68 mg/dl respectively.

The respective values in 25 mg/kg b.w. freeze dried aqueous extract treated group significantly reduced to 94.85±6.73, 24.214±5.43, 27.34±1.40, 136.73±5.89, 101.57±4.69, 333.15±17.31 and significantly increased to 43.29±3.27

The respective values in 50 mg/kg b.w. freeze dried aqueous extract treated group significantly reduced to 163.42±5.83, 97.626±5.17, 35.54±1.41, 177.72±5.81, 131.82±5.42, 472.96±17.06 and significantly increased to 30.25±2.64

### 5.3.3 Antioxidant and tissue protective effect

Freeze-dried aqueous extract ameliorated the diabetes induced tissue damage in major organs like liver, kidney, heart and pancreas through its antioxidant and tissue protective activity. Freeze-dried aqueous extract at both doses 25 or 50 mg/kg b.w shown significant inhibition (P<0.01) of raise in serum creatinine, BUN, ALT, AST and bilirubin. Freeze-dried aqueous extract at dose 50 mg/kg b.w significantly elevated the activity of superoxidedismutase, catalase and significantly reduced the lipid peroxidation in liver, kidney, heart and pancreas. At the dose 25 mg/kg b.w. shown the similar antioxidant activity except in heart Freeze-dried aqueous extract showed improved histological appearance of liver, kidney, heart and pancreas in dose dependent manner. The regeneration and rejuvenation of pancreatic beta cells was seen.
Hence the results from the present study demonstrated the antidiabetic,
antioxidant and hypolipidemic activity of freeze dried aqueous extract of *Murraya
koenigii* on intraperitoneal administration to alloxan diabetic rats.

5.4 EXPERIMENT-IV
5.4.1 Oral Glucose Tolerance Test

Compared to (FC) control group plasma glucose levels in G1 group
administered orally with aqueous extract at the dose of 800 mg/kg b.w. significantly
attenuated the raise in plasma glucose levels at 30 min 281.19±19.58 mg/dl and at 60
min 272.69±23.58 mg/dl. G2 group administered orally with methanol extract at the
dose of 800 mg/kg b.w. significantly attenuated the raise plasma glucose levels at 30
min 273.36±22.58 mg/dl and 263.58±19.51 mg/dl at 60 min. G3 group administered
intraperitoneally with freeze dried aqueous extract at the dose of 50 mg/kg b.w.
significantly attenuated the plasma raise in glucose levels at 30 min 271.49±21.67
mg/dl and 267.16±18.69 mg/dl at 60 min.

This study demonstrated the acute antihyperglycemic effect of *Murraya
koenigii* extracts. Kesari *et al*., 2005 reported a single oral administration of variable
dose levels (200, 300 and 400 mg/kg) of aqueous extract led to lowering of blood
glucose level in normal as well as in diabetic rabbits.

5.5 EXPERIMENT-V

The protein profile studies indicated the expression of additional proteins
expressed in pancreas of animals treated with aqueous and methanol leaf extracts of
*Murraya koenigii* at 800 mg/kg body weight. There was an over expression of
proteins between molecular weights 10 to 20 kDa.
Recent finding is that pancreas is having high plasticity and it can regenerate from existing damaged pancreas and also neogenesis. This happens by several cascade of cell signals that lead to the over expression of several specific proteins.

The endocrine portion of the pancreas, also called the islets of Langerhans, is distributed throughout the whole tissue. Exocrine pancreatic cells have been shown to be important in providing a compatible environment for the islets as they are in direct contact. Many studies have shown that the exocrine part of the pancreas secretes proteins that are important in the function and regeneration/replication of beta cells (Linghua et al., 2005).

Much work has been done to elucidate the function of the regeneration protein, reg I which is a 14 to 16 kDa protein. Reg protein appears to function as a growth factor in the pancreas, as well as its mitogenic effect on beta and ductal pancreatic cell lines (Zenilman et al., 1996) and its ability to reverse surgically induced diabetes (Terazono et al., 1990; Watanabe et al., 1994).

Transfection of the gene into the pancreatic β-cell line RINm5F cells resulted in increased mitogenesis after exposure to reg protein. Since reg and its receptor are linked to cellular mitogenesis and may affect repair of damaged pancreas (Bluth et al., 2006)

The present study demonstrated that the antidiabetic activity of aqueous and methanol extracts of Murraya koenigii was due to increased levels of insulin production through its ability to stimulate pancreatic β-cell regeneration and rejuvenation.

5.6 EXPERIMENT-VI

The repeated dose 28 days toxicity study conducted using aqueous and methanol extract did not reveal any significant changes in hematological, biochemical
and histological parameters at the tested doses 200 or 400 or 800 mg/kg b.w, as compared to saline control group at the tested doses.

The results in the present study corroborate the earlier studies by Khan et al., 1995., who reported *Murraya koenigii* diet fed rats did not show any histological abnormalities and haematological variations.

However in an earlier study reported by Adebajo et al., 2006., the *Murraya koenigii* methanol extract fraction from Nigeria reported to be moderately toxic with an LD50=316.23 mg/kg body weight in rats and had appreciable toxic effect on the liver and kidney at higher doses. They reported little or no effect on haematology and relative organ weight of lungs, heart and spleen. The variation in the toxicity could be due to phytochemical variations in the composition and concentration of *Murraya koenigii* grown in particular soil type and in particular climatic condition (Ranade et al., 2006). In the present study there were no toxicity symptoms, biochemical parameters, haemantological parameter or histological abnormalities. Both aqueous and methanol extracts of *Murraya koenigii* were found to be safe at the tested doses.

**5.7 PHYTOCHEMISTRY**

The petroleum ether extract of *Murraya koenigii* leaves showed the presence of steroids, triterpenes, alkaloids, flavonoid, tannins, diterpenes and saponins.

The chloroform extract showed the presence of steroids, alkaloids, flavonoids, tannins and glycosides.

The methanol extract showed the presence of steroid, alkaloids, flavonoids, tannins, lactones, diterpenes and saponins and the aqueous extract showed the presence of alkaloids, flavonoids, lactones, tannins and saponins.

Hence *Murraya koenigii* extracts have many phytochemical constituents, which are soluble in aqua and organic solvents.
From the present study on *Murraya koenigii* extracts it was concluded that:

a) Aqueous and methanol extracts were shown to be antihyperglycemic in action on oral administration

b) Aqueous and methanol extracts were shown to be antihyperlipidemic in action on oral administration

c) Aqueous and methanol were shown to possess antioxidant properties in terms of parameters estimated

d) Freeze dried aqueous extract on intraperitoneal administration was shown to be antihyperglycemic, antioxidant and hypolipidemic

e) The present study revealed that the antidiabetic action of constituents of *Murraya koenigii* extracts were could be due to stimulation of the existing pancreatic β-cell for the production of more insulin and also due to stimulation of pancreatic β-cell regeneration

f) From the present study it was found that aqueous and methanol extracts were safe over the repeated administration for 28 days in rats at the doses tested

It was also concluded that the multifaceted pharmacological activity of aqueous and methanol extracts of *Murraya koenigii* likely to be beneficial in the treatment of diabetes mellitus as they could bring glycemic control and prevent the diabetes induced complications.