Chapter 5

Discussion
Acute studies

Methanol and aqueous extracts of *P. granatum* and *N. nucifera* decreased the blood glucose of normal animals. In normal physiology glucose homoeostasis is maintained by two kinds of hormones, including insulin and counter-regulatory hormones (glucagon, growth hormone, cortisol and catecholamines) (Cryer and Polonsky, 1998; Pilkis and El-Maghrabi, 1988; Gerich, 1988). Despite the presence of such counter-regulatory hormones extracts produced hypoglycaemia, indicating that extract possess the pharmacological activity. But after 4 h the blood glucose levels started increasing, which indicates the counter-regulatory hormones overcame the hypoglycaemia produced by the extracts.

Theoretically, a plant with hypoglycaemic activity may act via the following fundamental mechanisms,

- at the intestinal level, by delaying or inhibiting glucose absorption (Anderson and Akanji, 1991; Adamson and Okafor, 1990)
- at the pancreatic level, by stimulating the secretion of insulin (Noor et al., 1989)
- at the peripheral level, by facilitating the entry of glucose into cells (Miura and Kato, 1995; Kato et al., 1995)
- at peripheral level by increasing the glucose utilisation and at hepatic level by decreasing the hepatic glucose production (Vats et al., 2004; Hanson and Reshef, 1997).

In this case, since the animals were fasted overnight before the start of the experiment, the action at the intestinal level by delaying or inhibiting glucose absorption can be ruled out.

In oral glucose tolerance test, the blood glucose levels in control animals rose to a peak value around 120-130 mg/100ml after 30 min of glucose load. In glucose tolerance, both methanolic and aqueous extracts of both the plants significantly
suppressed the peak rise of blood glucose after the glucose load. And they also brought the glucose levels near normal after 180 min which is comparable to that of standard drug glibenclamide.

The possible mechanism may be the extracts increased the peripheral glucose utilisation or at the pancreatic level by stimulating the secretion of insulin in response to increased glucose (Kar et al., 1999).

Administration of streptozotocin (STZ) destroys β-cells of the islets of Langerhans in pancreas (Elsner et al., 2000). Destruction of β-cells in the pancreas causes marked decrease in serum insulin levels (Gilman et al., 2001). The moderate action of standard drug glibenclamide in STZ-induced diabetic rats can be attributed to the presence of only negligible number of β-cells present in the pancreas to produce any secretogogue action by glibenclamide. However, methanolic and aqueous extracts of both *P. granatum* and *N. nucifera* exhibited good dose dependant antihyperglycaemic activity in STZ-induced diabetic rats and the percentage reduction of blood glucose levels are better than that of glibenclamide.

Since only a negligible number of β-cells survive the STZ challenge (Elsner et al., 2000) in diabetic animals, the possibility of extracts inducing insulin secretion to elicit the antihyperglycaemic activity can be ruled out. The possible mechanism may be either the extracts increase the glucose utilisation in the periphery or decrease the endogenous glucose production in the liver. The possibility of either one or more of these mechanisms with each extract contributed to the activity can not be ruled out at this stage. The mechanism via intestinal delay or inhibition of glucose can be ruled out as the animals used in the study were fasted overnight prior to the start of the experiment.

In all these three acute models, the chloroform extracts of both the plants were devoid of any antidiabetic activity. Only methanolic and aqueous extracts of *P. granatum* and *N. nucifera* showed antidiabetic activity. This indicates that the active constituents are polar in nature and present in the polar extracts such as the methanolic and aqueous and are completely absent in the non polar chloroform extract.

Jafri *et al.,* (2000) studied the antidiabetic activity of hydro-alcoholic extract of *P. granatum* flowers extract in alloxan-induced diabetic rats. Our study also showed
similar results with methanolic and aqueous extracts and is in confirmation with reports. Mukherjee et al., (1997) reported the antidiabetic activity of 80% ethanolic extract of N. nucifera rhizomes. In the present study, methanolic and aqueous extracts of N. nucifera flowers showed similar results.

Effect of 21 days administration of extracts on streptozotocin induced diabetes in rats

On the basis of the results we have got from the acute studies, methanolic extract of both plants at dose 500 mg/kg were used in chronic STZ-induced diabetic study. In STZ-diabetic rats the deficiency of serum insulin has been well documented (Wohaieb and Godin, 1987). In the present study, serum insulin levels of diabetic control rats were markedly decreased when compared with normal rats. Thus the role of endogenous insulin is negligible in STZ-induced diabetic rats.

The results indicate that the continued administration of the extracts of P. granatum and N. nucifera produced a sustained antihyperglycaemic effect in STZ-induced diabetic rats. STZ-induced diabetes in rats represents the IDDM. The ability of extracts to reduce the blood glucose levels in these animals when compared with normal untreated rats further substantiates an extra-pancreatic mode of action of the extracts. The decrease of blood glucose was more when compared with that of corresponding acute treatment and was marked on day 21.

In the present study serum insulin levels of diabetic control rats were found to be markedly decreased when compared with normal control rats. Treatment with extracts of P. granatum and N. nucifera did not increase the insulin levels significantly. This indicates that the extracts did not induce any regeneration of pancreatic β-cells. They did not induce the insulin secretion to produce the antihyperglycaemic action. But the insulinotropic action of the extracts can not be ruled out, because only a negligible number of β-cell would have been available for any insulinotropic action if any, of the extracts.

P. granatum flower extract is reported to be a potent α-glucosidase inhibitor and improved the postprandial hyperglycaemia in Zucker diabetic fatty rats (Li et al., 2005). Thus the presence of α-glucosidase inhibitor in P. granatum extract can be attributed to the significant decrease of non-fasting blood glucose levels in 21 day
study. But however, *P. granatum* extracts decreased blood glucose levels in normal fasted and STZ-induced diabetic fasted rats, indicating the presence of more than one active component with different mechanisms.

Treatment with STZ decreased the activity of liver hexokinase enzyme when compared with that of normal control animals. Liver phosphoenolpyruvate carboxykinase (PEPCK) activity was found to be higher in untreated diabetic animals when compared with normal control animals. This is in agreement with previously reported studies. STZ-diabetic animals with lower level of activity of hexokinase have been already reported (Vats *et al.*, 2004).

Hexokinase is a key enzyme in glycolytic pathway. It converts glucose into glucose-6-phosphate in the glycolysis process. Insulin deficiency in chemical models of diabetes (STZ and alloxan induced diabetes) causes suppression of hexokinase activities (Grover *et al.*, 2000; Rathi *et al.*, 2002; Raju *et al.*, 2001). Administration of insulin in these animals caused normalization of enzymatic activities (Weber *et al.*, 1966). All these imply that the measurement of hexokinase may be indicative of glucose utilization.

PEPCK is a key enzyme involved in gluconeogenesis in liver (Barthel and Schmoll, 2003). Studies in diabetic animals showed that augmented gluconeogenesis is a major factor in the increased plasma glucose that appears in fasting and postprandial states (Hanson & Reshef, 1997). Insulin directly inhibits the gluconeogenesis. Since only a negligible quantity of insulin is secreted in the STZ-induced diabetic animals, the suppression of gluconeogenesis by insulin becomes minimal. This could be the reason for higher levels of the gluconeogenic enzyme PEPCK in the STZ-diabetic animals. mRNA levels of PEPCK in liver from the STZ-induced diabetic rats was 1.7 fold of that in liver of normal rats (Cheng *et al.*, 2001; Liu *et al.*, 2000).

In the present study, liver hexokinase activity was found to be decreased in diabetic control animals when compared with the normal animals. Treatment with the extracts of *P. granatum* and *N. nucifera* increased the activity of liver hexokinase enzyme in STZ-diabetic animals. This indicates the extracts were effective in increasing the utilization of glucose. The increased utilization of glucose by the extracts treated
STZ-diabetic animals could be one of the possible mechanisms of action of their antihyperglycaemic activity. The activity of gluconeogenic enzyme PEPCK was found to be increased in the STZ-induced diabetic rats when compared with that of normal control rats. Groups treated with the extracts of *P. granatum* and *N. nucifera* showed lower levels of PEPCK activity when compared with the untreated STZ-diabetic rats. This indicates the gluconeogenesis was suppressed. Gluconeogenesis significantly contributes to the endogenous hepatic glucose production. This can contribute to the antihyperglycaemic action of the extracts. Since the extracts also increased the glucose utilisation by increasing the enzyme hexokinase, this can add to their antihyperglycaemic action.

Diabetic conditions are associated with the elevated lipid levels along with the hyperglycaemia (Brown and Goldstein, 1994; Jay and Betteridge, 1994). Levels of plasma triglycerides and cholesterol in individuals in various types of diabetes are higher that that of normal subjects (Chase & Glasgow, 1976). However the level of lipids depends on the severity of diabetes, its therapeutic control and the composition of diet. It has been known that dietary ingredients affecting glucose metabolism may also influence the lipid metabolism (Jayasooriya *et al.*, 2000; Jenkins *et al.*, 1995). Lipid profile, which is altered in the serum of diabetic patients (Betteridge, 1994), appears to be a significant factor in the development of premature atherosclerosis and includes an increase in triglyceride and total cholesterol levels.

Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia. In insulin deficiency diabetes, the plasma free fatty acids concentration is elevated as a result of increased free fatty acid outflow from the fat depots (Chase and Glasgow, 1976). The low capacity for triglyceride storage can be attributed to the low activity of lipoprotein lipase resulting from insulin deficiency, since its synthesis is inducted by insulin (Ghosh and Suryawanshi, 2001; Garfinkel *et al.*, 1976).

In experimental diabetes, administering streptozotocin produces insulin deficiency. It produces hyperglycaemia as well as dyslipidemia as indicated by the rise of
triglycerides, LDL and VLDL (Shein et al., 1971). The liver and some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoproteins.

Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications (Betteridge 1997). Many herbs and plant products have been shown to have antihyperglycemic and antihyperlipidemic property (Brown et al., 1993).

Results indicate that the induction of diabetes in rats with streptozotocin elevated the circulating lipid levels. Serum cholesterol levels and various fractions of lipoproteins such as LDL, HDL, VLDL and triglycerides were found to be increased when compared with normal control rats. The atherogenic indices of STZ-diabetic rats were found to be higher than the normal rats. The atherogenic index represents the ratio of the LDL and VLDL to HDL. Higher the value higher will be chances of atherogenesis which might lead to cardiovascular complications as well. Treatment with insulin brought down the levels of increased lipoproteins and total cholesterol. Administration of extracts of *P. granatum* and *N. nucifera* also decreased the levels of circulating lipids. They also lowered the atherogenic index which indicates the balance of lipoprotein fractions in favour the beneficial HDL. *P. granatum* decreased the atherogenic index more effectively and the increase in HDL was found to be higher.

Induction of diabetes with STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes due to unavailability of carbohydrate for utilization as an energy source (Swanston-Flatt et al., 1990). In the present study there was gradual increase in body weight in the normal controls while the diabetic controls continued to lose weight. This is in agreement with earlier reports (Prince et al., 1998; Yadav et al., 2002).

Treatments with the extracts of *P. granatum* and *N. nucifera* decreased the reduction in body weight by diabetes. The treatments altered the body weights of diabetic animals towards normalcy. This may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis which also evidenced by the
decreased levels of activity of the enzyme PEPCK in the animals of extract treated groups.

The standard reference treatment with insulin also decreased the weight loss of diabetic animals and altered the weight towards normalcy during the course of treatment.

Effect of 21 days administration of extracts on streptozotocin-nicotinamide induced Diabetes in rats

The partial protection exerted by suitable dosage of nicotinamide against the beta-cytotoxic effect of STZ is used in a new experimental diabetic syndrome in adult rats that appears closer to NIDDM than other available animal models with regard to insulin responsiveness to glucose and sulfonylureas (Pellegrino et al., 1998)

The STZ and Nicotinamide induced diabetic rat is a mild diabetic animal without associated obesity and is primarily characterized by reduced pancreatic stores (~40% of normal) and defective insulin secretion which presents a good stability of diabetes (Broca et al., 1999). This model is particularly suitable to investigate the potential antidiabetic properties of new insulinotropic agents.

The results indicate that administration of nicotinamide fifteen minutes after the administration of streptozotocin produced a moderate hyperglycemia in rats. The extracts of P. granatum and N. nucifera decreased the blood glucose levels significantly when compared with the diabetic control animals. Since the extracts were able to decrease the blood glucose in STZ induced diabetic animals, the same mechanisms would also apply in this model. But since in this study the nicotinamide would have protected at least 40% of pancreatic β-cells and these would have been available for the extracts to exert the insulinotropic action if any they have.

Serum insulin levels show that the diabetic control animals had decreased levels of serum insulin but not as that of STZ induced diabetic rats. Treatment with extracts of P. granatum and N. nucifera did not increase the serum insulin levels significantly when compared with that of untreated diabetic control rats whereas, the reference drug glibenclamide produced a significant increase in serum insulin levels but not near the normal levels. The reason may be either because the surviving β-cells simply not sufficient in number or the short plasma half-life of glibenclamide as well as
insulin. But however, treatment with glibenclamide produced near normal levels of blood glucose when compared with diabetic and normal control rats.

Serum insulin sensitivity can be estimated using insulin tolerance test (ITT). This test measures insulin sensitivity using $K_{ITT}$ as an index of insulin mediated glucose metabolism. ITT which represents the response to exogenously administered insulin on blood glucose has been used to estimate insulin sensitivity (Alford et al., 1971). Results indicate that the STZ and nicotinamide induced diabetic animals did not show any altered sensitivity to insulin and their insulin mediated glucose disposal remained almost normal when compared with untreated normal rats.

Insulin deficiency is associated with hypercholesterolemia and hypertriglyceridermia (Durrington, 1993). STZ-diabetes is reported to have increased plasma levels of cholesterol, triglyceride, free fatty acid and phospholipids (Rodrigues et al., 1986). Insulin deficiency may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol rich LDL particles. The mechanisms responsible for the development of hypertriglyceridermia in uncontrolled diabetes in humans (possibly in insulin deficient STZ-diabetic rats) are due to a number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver (Balasse et al., 1972). With longer insulin deficiency liver converts free fatty acids into ketone bodies and VLDL-triglyceride secretion diminishes (Basso and Havel, 1970). At the same time, lipoprotein lipase activity falls (Nikkila et al., 1977) resulting in impaired clearance of VLDL and chylomicronmes from plasma (Bagdade et al., 1968).

In our study also the STZ and nicotinamide induced diabetic rats showed hypercholesteremia and hypertriglycerideremia. Treatment with extracts of *P. granatum* and *N. nucifera* decreased the increased levels of serum cholesterol and triglycerides. They also altered atherogenic indices in favour of HDL which would be beneficial in reducing incidences of cardiovascular complications.

Hexokinase is a key enzyme in glycolytic pathway. It converts glucose in to glucose-6-phosphate in the glycolysis process. As described already, Insulin deficiency in
chemical models of diabetes (STZ and alloxan induced diabetes) causes suppression of hexokinase activities (Grover et al., 2000; Rathi et al., 2002; Raju et al., 2001).

Results indicate that in the present study, liver hexokinase activity was found to be decreased in diabetic control animals when compared with the normal animals. Administration of the extracts of *P. graminatum* and *N. nucifera* increased the activity of liver hexokinase enzyme in STZ-nicotinamide diabetic animals. This substantiate that the extracts can increase the utilization of glucose. The increased utilization of glucose by the extracts treated STZ-nicotinamide diabetic animals could be one of the possible mechanisms of action of their antihyperglycaemic activity.

As mentioned already, PEPCK is a key enzyme involved in gluconeogenesis in liver (Barthel and Schmoll, 2003). Insulin directly inhibits the gluconeogenesis. Since there is a significant deficiency in insulin in the STZ-nicotinamide induced diabetic animals, the suppression of gluconeogenesis by insulin becomes insufficient enough to control the gluconeogenesis. This could be the reason for higher levels of the gluconeogenic enzyme PEPCK in the STZ-nicotinamide diabetic animals.

In the present study, the activity of gluconeogenic enzyme PEPCK was found to be increased in the STZ-nicotinamide induced diabetic rats when compared with that of normal control rats. Groups treated with the extracts of *P. graminatum* and *N. nucifera* showed lower levels of PEPCK activity when compared with the untreated STZ-nicotinamide diabetic rats. This indicates that the gluconeogenesis was suppressed. Since gluconeogenesis significantly contributes to the endogenous hepatic glucose production, suppression of gluconeogenesis can contribute to the antihyperglycaemic action of the extracts. As the extracts also increased the glucose utilisation by increasing the enzyme hexokinase, both can add to their antihyperglycaemic action.

Induction of diabetes with STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes due to unavailability of carbohydrate for utilization as an energy source (Swanston-Flatt et al., 1990). In this study since only a part of β-cells have been destroyed, as evidenced by the lower levels of serum insulin in diabetic control rats, the muscle wasting could have been lower than the only STZ induced diabetic rats. This can be clearly seen from the extent of body weight of STZ-nicotinamide diabetic control rats when compared with
that of STZ-diabetic control rats and also with that of normal control rats. Since both 
P. granatum and N. nucifera extracts increased the glucose utilisation, there is a possibility that the extracts decreased the extent weight loss by decreasing the muscle wasting.

Effect of 21 days administration of extracts on High Fat Fed Insulin Resistant Rats

Rats fed on a high fat diet (60% of calories as fat) develop insulin resistance with reduced basal glucose metabolism (Kraegen et al., 1986). There is increasing evidence that lipid accumulation in muscle and liver leads to development of insulin resistance (Zierath et al., 1997; Chalkley et al., 1998; Griffin et al., 1999). Reduction of obesity or lowering of lipids generally improves insulin sensitivity (Hansen et al., 1997; Markovic et al., 1998). Studies suggest that thiazolidinediones improve muscle insulin action by sequestering lipids in adipocytes, a mechanism that ultimately reduces lipid accumulation in muscle (Spiegelman, 1998; Kersten et al., 2000).

High fat diet fed Wistar rats develop insulin resistance in 2 weeks (Ye et al., 2001). In the present study, in rats fed on high fat diet a mild, but significant increase in blood glucose was observed. Development of hyperglycaemia in high fat fed rats take very long time (Kraegen et al., 1986). With the development of insulin resistance, the peripheral insulin mediated glucose disposal (Storlien et al., 1986) and inhibition of hepatic glucose production (Goldstein, 2002) are affected resulting in hyperglycaemia in high fat fed rats. Treatment with the P. granatum decreased the blood glucose significantly and is more effective than the standard reference drug rosiglitazone. N. nucifera extracts did not produce any change in the blood glucose levels. Both extracts were effective in decreasing blood glucose in normal as well as streptozotocin-induced diabetic animals. But only P. granatum was found to be effective in this insulin resistance study, which indicates that P. granatum might have increased the insulin sensitivity.

The activation of PPAR-γ receptors is the mechanism of action of the standard reference rosiglitazone (Kersten et al., 2000). Direct activation of PPAR-γ leads to contribution of lowering triglyceride and fatty acid levels and suppress TNF-α gene expression, which is potential systemic mediator of insulin resistance (Moller, 2001).
Activation of PPAR by *P. granatum* in high fat fed rats might have lead to the improvement of insulin resistance with the consequence that plasma glucose levels were lowered.

Insulin tolerance test (ITT) (Alford *et al.*, 1971) is used to assess peripheral insulin resistance. This test measures insulin sensitivity using $K_{\text{ITT}}$ as an index of insulin mediated glucose metabolism. ITT represents the response to exogenously administered insulin on blood glucose has been used to estimate insulin sensitivity (Alford *et al.*, 1971). ITT is a simple, reasonably accurate and rapid method for screening insulin resistance (Gruel *et al.*, 1993). ITT indicates net result of resistance to insulin action at target level including receptor and post receptor defects. Results show that high fat fed animals had higher $T_{1/2}$ (glucose) and a lower $K_{\text{ITT}}$ when compared with normal control rats indicating that the insulin mediated glucose disposal is impaired. This indicates the development of insulin resistance in high fat fed rats.

Administration of *P. granatum* extract decreased the $T_{1/2}$ and increased the $K_{\text{ITT}}$. This further substantiates the possibility of *P. granatum* increasing the insulin sensitivity. *P. granatum* flowers extract was reported to contain active constituents which activate PPAR-γ receptors (Huang *et al.*, 2005). The activation of PPAR-γ receptors is the mechanism of action of the standard reference rosiglitazone (Kersten *et al.*, 2000). This clearly indicates that increased insulin sensitivity through the activation of PPAR-γ receptors can be the possible mechanism of action of *P. granatum* flowers extract. Whereas, *N. nucifera* extract did not alter the $T_{1/2}$ and $K_{\text{ITT}}$. This indicates that *N. nucifera* extracts might not be effective in the presence of insulin resistance.

The results of serum insulin levels show that high fat diet fed animals were hyperinsulinemic confirming the presence of insulin resistance. Treatment with standard drug rosiglitazone brought back the increased serum insulin levels. Treatment with *P. granatum* extract also decreased the serum insulin levels significantly when compared with high fat fed control rats, indicating similar mechanism of action. This further substantiates that the activation of PPAR-γ receptors could be the possible mechanism of action of *P. granatum* extract. Administration of *N. nucifera* extracts did not lower the increased insulin levels when
compared with the high fat fed control group indicating that *N. nucifera* extract is ineffective in controlling the insulin resistance.

Hexokinase is a key enzyme in glycolytic pathway. It converts glucose into glucose-6-phosphate in the glycolysis process. There is evidence of reduction in insulin-mediated glucose metabolism occurs among rats fed on high fat diet (Storlien *et al.*, 1986). The results of the present study shows that the activity of hexokinase enzyme in high fat fed rats is significantly lower than that of normal diet fed rats, indicating that suppression of glycolysis occurs. Improvement in insulin sensitivity by the standard reference drug rosiglitazone increased the activity of hexokinase enzyme significantly.

Treatment with *P. granatum* extract also increased the hepatic hexokinase activity levels significantly, indicating an improvement in insulin sensitivity. Administration of *N. nucifera* extract did not increase the levels of hexokinase enzyme activity. This clearly indicates that *N. nucifera* did not improve the insulin sensitivity in high fat diet fed rats.

High free fatty acid concentrations in liver contribute to the resistance to the action of insulin by enhancing glucose output from liver (Goldstein, 2002). Accumulation of triglycerides by the high fatty acid concentrations also bring about non alcoholic fatty liver disease, which damages the liver, eventually leading to altered glucose metabolism, since liver is the main organ of glucose metabolism in the body (Angulo, 2002). PEPCK is a key enzyme involved in gluconeogenesis in liver (Barthel and Schmoll, 2003). Insulin directly inhibits the gluconeogenesis (Cheng *et al.*, 2001). Since the insulin sensitivity is altered in the high fat diet fed rats, the inhibition of gluconeogenesis by insulin can be expected to be lesser than in normal animals. The results show that the PEPCK enzyme activity was significantly higher than that of normal diet fed animals, indicating that the inhibition of gluconeogenesis in high fat fed rats by insulin has become lesser.

Treatment with standard drug rosiglitazone, which improves the insulin sensitivity through its PPAR-γ receptor agonist action, brought back the increased levels of enzyme PEPCK in high fat fed animals. *P. granatum* which is also reported to have PPAR-γ receptor agonist action (Huang *et al.*, 2005), decreased the level of PEPCK
enzyme activity of high fat diet fed rats when compared to that of normal diet fed rats. Thus the PPAR-γ receptor agonist action of *P. granatum* could be safely attributed to its insulin sensitising activity seen in high fat diet fed rats.

The results show that plasma lipid levels increased in high fat fed drug when compared with rats on normal diet. This compares well with the earlier findings in high fat fed animals (Park *et al.*, 2005). A high fat intake and the resulting increased circulation of free fatty acid might lead to insulin resistance and ultimately to diabetes mellitus in susceptible cases by the mechanism of lipotoxicity (Manco *et al.*, 2004). Standard reference drug rosiglitazone improved the plasma lipid levels significantly. Direct activation of PPAR-γ leads to contribution of lowering triglycerides and free fatty acid levels (Moller, 2001). As *P. granatum* is an agonist of PPAR-γ receptor (Huang *et al.*, 2005), it appears the activation of PPAR-γ contributed to the improvement of lipid lowering by *P. granatum*. Administration *N. nucifera* extract also improved the plasma lipid profile favourably. But since it did not improve other parameters such as serum glucose, serum insulin levels and liver enzymes, it appears that *N. nucifera* might have lowered the increased plasma lipid by an independent mechanism.

There was approximately a 10% increase in body weight of high fat fed animals when compared to that of normal diet fed animals. This increase is higher than that occurred in normal diet fed rats during the study. Fat feeding is reported to cause obesity in animals (Storlien *et al.*, 1986). The increased weight of high fat fed animals can be attributed to the accumulation of triglycerides and fatty acids in adipose tissue and skeletal muscle.