Body weight

The normal control group in which diabetes was not induced gained an average weight during the period of study. The diabetic control group which was not given any treatment other than the vehicle did not gain any weight.

The breakdown of structural proteins is known to contribute to body weight, thus the decrease in the body weight in the rats induced with diabetic is clearly related to loss or degradation of these structural proteins. The body weight could also be decreased due to dehydration and catabolism of fats (Hakim ZS et al, 1997) as well as proteins. Gain in the body weight is an indicator of efficient glucose homeostasis.

In diabetics, the body cells scarcely access glucose, and on the alternative, fats and tissue proteins are broken down for energy supply (muscle wasting) accounting for loss in body weight (Atangwho IJ et al, 2012). Loss of body fluids and body weight has also been linked with diabetes mellitus (Pupium LB et al, 2005). Ravi K et al (2004) reported that excessive break down of tissue proteins and increased muscle wasting are specific reasons for loss of body weight in streptozotocin induced diabetes. Decreased fasting blood sugar improves body weight in alloxan induced diabetic rats (Nagarajan NS et al, 2005;Pari L et al, 2004).

Dhanraj et al (2007) reported that the production of protein is not favored when there is deficiency of insulin. Additionally, several studies have also reported that the levels of
serum total protein were declined in diabetic animals (Surana SJ et al, 2008; Yokozawa T et al, 2008; Gupta R et al, 2009).

*Sesamum indicum* plant extract prevented the loss in body weight which may be due to increasing glucose uptake in peripheral tissues or by inhibiting catabolism of fat and protein or by glycemic control.

**Blood glucose**

Oral administration of the *P. marsupium* extract showed significant decrease in the blood glucose level, decline in total cholesterol and triglyceride which may be an indication of the progressive metabolic control of the extract on mechanisms involved in the elimination of lipids and glucose from the body. Similar hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use (Kono et al, 1992; Naidu and Thippeswamy 2002; Devi and Sharma 2004). The most extreme form of diabetes mellitus would be deficiency of insulin and/or the increase in counter insulin hormones. The increase in the latter would be very severe to increase hepatic glycogenesis well beyond the fuel need of the patient. The ability of the peripheral tissues to utilize glucose is impaired leading to glycosuria.

It is likely that the plant extract produces its hypoglycemic effect by acting as an analog of insulin and it also mimics some of the actions of insulin on glucose metabolism such as, enhancing uptake of glucose absorption in the intestine as well as acting as antimetabolites that are capable of blocking the pathway of fatty acid oxidation. The results of the present study is in accordance with the previous study which demonstrated that the aqueous extract of *Mangifera indica* leaf at a dose of 400mg/kg body wt reduced the blood
glucose level significantly in alloxan induced diabetic rats which indicates that alloxan induces diabetes by completely destroying the pancreatic islet of beta cells which produces insulin.

Presence of flavonoids could also be a probable cause for producing hypoglycemia as supported by previous studies (Rauter AP et al, 2009; Wang HX and Ng TB, 1999).

**Serum cholesterol and triglyceride**

Diabetes in general is associated with accelerated atherosclerosis and predisposes to certain microvascular complications. Elevated total cholesterol is having an equal atherogenic potential in diabetic as in non diabetic subjects. The liver, insulin dependent tissue plays a vital role in glucose and lipid homeostasis, is severely affected during diabetes.

In diabetes, enhanced activity of the enzyme hormone-sensitive lipase increases lipolysis and releases more free fatty acids into circulation. Increased fatty acid concentration and increase beta oxidation of fatty acids produces more acetyl-CoA and cholesterol in diabetics.

The hypercholesterolemia observed in diabetics generally might be due to increased intestinal cholesterogenesis, resulting from increased activity of HMG-COA reductase (β-OH-β methyl glutaryl COA) in the intestine of alloxan induced diabetic rats as reported by H Nakayama and S Nagakawa (1977) partly from the increased availability of acetyl-CoA as a result of increased oxidation of fatty acids in diabetes mellitus. The fall in the serum cholesterol level of diabetic rats that received the plant extract further supports the hypoglycemic effect of *P.marsupium*
The plasma lipid level is usually raised during diabetes and enhances the risk factor for the coronary heart disease and lowering lipid level indicates the decrease in the risk of vascular complications.

In the present study there was an decrease in the cholesterol level in DE and PE treated groups indicating that, the hypocholesteremic effect may be an indication of progressive metabolic control of the plant extract. The fall in the cholesterol level of alloxan induced diabetic rats that received plant extract further supports the hypoglycaemic effect of the heart wood of *P. marsupium*.

Similar hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use (Kono et al, 1992).

In the present study, it was found that the diabetic control group was able to register hypotriglyceridemia even though there could not be any significant increase or decrease in other extract treated groups.

A reduction in TG levels may be due to decreased lipogenesis and increased lipolytic activation of the hormone sensitive lipase (Al-Shamaony I et al, 1994) or lipogenic enzymes (Pari L et al, 2004), and/or activation of lipoprotein lipase (Ahmed I et al, 2001), as is observed in antidiabetic plants such as (Ahmed I et al, 2004) Ormodica charantia. However, decrease in cholesterol and triglyceride levels indicates that the plant extract is more useful in the treatment of diabetes as it has hypolipidemic effect.

As many antidiabetic drugs do not correct dyslipidaemia, the observed hypocholesterolaemic and hypotriglyceridaemic effects of the extract in alloxan induced diabetic rats unveils the potential effects of *P. marsupium* in the management of diabetes because the
extract may reverse dyslipidaemia associated with diabetes and prevent cardiovascular complications. Similar observations have also been proved in the hydroalcoholic extract of \textit{P.dactylifera} plant (Nagarajan NS et al, 2005).

Administration of ethanol extract of the leaf of \textit{Gynura} procumbens (Zhang and Tan, 2000), seeds of \textit{Eruea} saliva (Missiry EL et al, 2000), ethanol extract of leaves of \textit{Averrhoa hilimbi} (Pushparaj et al, 2000) etc., have also demonstrated the hypotriglyceridemic effects in diabetes.

**HDL, LDL**

LDL and VLDL carry cholesterol to peripheral tissues where it is deposited; hence increased levels of LDL and VLDL are atherogenic. HDL transports cholesterol from the peripheral tissues to the liver and thus aids in its excretion. HDL therefore has a protective effect.

Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver. Increased LDL cholesterol may be due to over production of VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx or decreased removal of VLDL and LDL from the circulation or by inhibiting receptor mediated removal of LDL (Tsustumi et al, 1995).

Diabetic dyslipidaemia is mainly due to decreased removal of triglycerides into the fat depots and the increase in the plasma concentration of LDL-cholesterol.

However, in the present study there was no significant change in HDL levels in any extract treated groups compared to DC which indicates a low risk factor for atherosclerosis. Earlier
studies by (Laakso 2001 and Taskinen MR, 1992) showed higher concentration of LDL cholesterol and lower concentration of HDL cholesterol in diabetic patients.

**Urea, uric acid, creatinine**

Distinct metabolic alterations are demonstrable in experimental diabetic rats, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Bhavapriya V et al, 2001). Changes in the protein metabolism could be due to the following mechanisms:

1) Reduced uptake of amino acids by tissues.
2) Higher rate of proteolysis
3) Fall in protein synthesis

All these factors lead to increased production of urea by liver as reported by (Felig P et al, 1995). One of the most important sensitive and dramatic indicators of kidney injury is the increase in serum levels of urea and creatinine. Plasma uric acid and creatinine can be used as a rough index of glomerular filtration rate (Hermandz T et al, 1967).

Increase in uric acid may be due to metabolic disturbance in diabetes, which is reflected in high activities of xanthine oxidase, lipid peroxidation and increased cholesterol and triglycerides (Madinov et al, 2000; Anwar and Meki 2003). Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase (Anwar and Mek 2003). Increase in serum creatinine level is also the marker of muscle wastage. The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine and uric acid which are considered as significant markers of renal dysfunction (Almdal m et al, 1987). However, in the present
study, there was no increase in urea, uric acid and creatinine levels among all the experimental groups which demonstrates that the plant extract is safe to the kidney.

**Glycogen**

Liver glycogen level may be considered as the best marker for assessing hypoglycemic activity of any drug. This indicates that the peripheral free glucose is being stored in the liver in the form of glycogen by increasing glycogenesis. During starvation and stress, one of the fuel reserves broken down to provide energy is glycogen. The principal reservoir of glycogen from which free glucose can be released into circulation is liver.

Administration of alloxan causes decrease in glycogen content due to enhanced glycogenolysis, which is due to insulin deficiency so that the normal capacity of the liver to synthesize glycogen is impaired (Dheer R, Bhatnagar P, 2010).

A significant increase in the liver glycogen by administration of Mollugo nudicaulis, may be due to an increase level of insulin by it.

In the present study, there was an increase in the glycogen content in the insulin and alcohol treated groups in comparison with diethyl ether treated group which could be due to disturbances in glycogen synthetase system. Liver glycogen changes may require higher doses of the extract and are not observed at the lower doses. Decrease in glycogen content in streptozotocin induced diabetic rats support the findings of Grover et al (2002).

**Protein thiols**

The measurement of plasma thiol is a good reflection of excess free radical generation, since the conformation of albumin is altered showing SH groups to be oxidized (Cakatay U, ...
Kayali R, 2005; Sedlak J, Lindsy RH, 1968). It has already been shown that free radicals cause oxidation of protein SH groups in plasma. Albumin is the most abundant plasma protein and is a powerful extracellular antioxidant.

In diabetes mellitus, hyperglycemia can simply inactivate antioxidant enzymes such as SOD and GPX by glycating these proteins and induces oxidative stress which in turn causes lipid peroxidation (Vincent AM 2004; Kaleem M et al, 2006). Decreased antioxidant enzymes levels and enhanced lipid peroxidation have been well documented in alloxan induced diabetes (Stepici-Dincel A et al, 2007; Oyedemi S et al, 2011; Mohammadi S et al, 2010).

The PE, EA and aqueous treated group had a statistical significant decrease in protein thiols compared to alcohol treated group. Administration of the extract has caused significant reduction in blood glucose and a reduction in protein thiol. It indicates that, there is an improved glycemic control in these rats which accounts for the lowering of protein thiols in these groups as an adaptive response. Decrease in total thiols would probably represent increased utilization for neutralizing free radicals.

Phytochemical constituents would also be responsible for antioxidant activities which suggests that the plant extract possess free radical scavenging nature.

**Phytochemical constituents**

The alcoholic extract of the heart wood of *P. marsupium* showed the presence of saponins, triterpenes, tannins and flavonoids which could also be a probable cause for the antidiabetic effect of our extract. The presence of flavonoids in the plant regenerate the damaged beta cells of pancreas and saponin present in the plants inhibit glucose transport by inhibiting sodium glucose co-transporter (GLUT-1) in intestine (Hakkim FL et al, 2011; Tiwari AK et
Saponins found in the plant extracts are suggestive of their antihyperlipidemic properties. Previous studies have shown that saponins have hypocholesterolemic activities (Oakenfull D, 1996). One of the previous study also reported the presence of flavonoids, saponins and polyphenolic compounds in the ethanolic extract of the whole plant of Tridax procumbens which had antidiabetic activity.

The antioxidant activity of the phenolics, tannins and flavonoid compounds are attributed to its redox properties which can act as reducing agents, hydrogen donators, and single oxygen quenchers (Gulcin I et al, 2007). Polyphenolics having hydroxyl groups are very important plant constituents which can protect from oxidative stress (Jing LJ et al, 2010).

**Antimicrobial action**

Phytochemical extracts from plants holds promise to be used in allopathic medicine as they are potential sources of antiviral, anti-tumoral and antimicrobial agents (Nair et al, 2005).

In our present study, we found a promising antimicrobial activity towards both Gram +ve and –ve test organism at 100µg of the alcoholic extract.

In this study, the agar diffusion test was used to check the antimicrobial efficacy of the test medicaments. The generally accepted method to test the antimicrobial activity of plant extracts is the agar diffusion test. The test is relatively inexpensive and is standardised making it reproducible and simple to perform. However, certain factors such as the pH of the substrate, incubation period, toxicity, sensitivity and diffusion capacity of the drug may also have an impact on the antimicrobial activity of the test materials in the plates.
In our study, the presence of saponins, flavonoids contributed to the antimicrobial action. Saponins have been reported to possess a wide range of biological activities. The antifungal, antiviral and antibacterial activities are well documented (Lacaille-Dubois and Wagner 1996; Milgate and Roberts, 1995). The mode of action of antibacterial effects of saponins seems to involve membranolic properties, rather than simply altering the surface tension of the extracellular medium, thus being influenced by microbial population density (Killen et al, 1998).

Flavonoids are known to be synthesized by plants in response to microbial infection (Dixon et al, 1983). The antimicrobial action of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al, 1996).

The antifungal activity may be attributed to various chemicals detectable in its extracts such as saponins (Zhang et al, 2006). The mechanism of action of saponins may be due to the damage of the cellular membrane and leakage of cellular materials, ultimately leading to cell death (Mshvildadze et al, 2000).