Diabetes is a common and very prevalent disease worldwide. India is much affected with diabetes epidemic and is predicted to be the "diabetic capital of the word". Common medicinal plants play important role in the management of diabetes especially in developing countries where resources are meager and public perceives natural products are safe. Diabetes is a common and very prevalent disease affecting the peoples of both developed and developing countries. It is estimated that around 25% of the world population is affected by this disease [Erasto et al., 2005]. The major types of diabetes are characterized by hyperglycemia, abnormal lipid and protein metabolism, along with specific long-term complications and irreversibly affect the retina, liver and kidney [David et al., 1997]. Hyperglycemia is an important factor in the development and progression of the complications of DM, and good glycemic control is necessary to prevent diabetic complications [Luzzi, 1998].

Sulphonyl urea and a few biguanides are valuable treatment for hyperglycemia, but they are unable to lower glucose level to within normal range and reinstate a normal pattern of glucose homeostasis permanently. Use of these therapies is restricted by their pharmacokinetics properties, secondary failure rates and accompanying side effects [Melinda, 1988]. Even insulin therapy does not reinstate a permanent normal pattern of glucose homeostasis, and carries an increased risk of atherogenesis and hypoglycemia. Therefore, WHO has recommended that plant research warrant attention [WHO report, 1980].

Medicinal plants have the advantage of having none or only a few side effects. Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world. But only a few have been evaluated as per modern system of medicine. From many such plants, only extracts have been prepared and their usefulness evaluated in experimental diabetes in animals. Most of them seem to act directly on tissues liver, muscles etc. and alter favorably the activities of the regulatory enzymes of glycolysis, gluconeogenesis and other pathways. Many of its products/chemical constituents are known to possess wide array of medicinal properties.

Our results show significant changes in physiological and biochemical parameters during experiments on diabetes within short period. Apart these changes, various histopathological changes in liver and pancreas tissues after 4 weeks treatments were revealed. We demonstrate the efficacy of naturally occurring herbal extract Butea monosperma, Terminalia arjuna and Pycnogenol in preventing/reverting diabetes induced biochemical and histopathological alteration in liver and pancreas of diabetes rats.
STUDY ON TYPE 1 DIABETES

Experiment I: The present study was design to evaluate the beneficial effects of *Butea monosperma* (BM) extract on hyperglycemia, lipid profile, renal damage markers, inflammatory and oxidative stress in the liver and the pancreas of type 1 diabetes mellitus (T1DM) in rats and compared to a standard drug, glibenclamide (GL). T1DM was induced by single dose of streptozotocin (STZ; 60 mg/kg, intraperitoneally) followed by 5% oral glucose for 24 hr. Control and diabetic rats were treated with BM (300 mg/kg) for four weeks. After BM treatment, blood was drawn and rats were then sacrificed, and their liver and pancreas were dissected for biochemical and histopathological assays. The level of fasting blood glucose, glycated hemoglobin, total cholesterol, triglycerides, free fatty acids, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol significantly increased while high density lipoprotein cholesterol, amylase, insulin and hepatic glycogen decreased in the STZ group. BM treatment augmented these effects in the STZ + BM group. The STZ group showed elevated renal injury markers in serum, including blood urea nitrogen, serum creatinine and alkaline phosphatase, which were decreased significantly by BM treatment. STZ group showed increased level of nitric oxide, tumor necrosis factor-α and interleukin-1β in serum which were modulated by BM treatment. Moreover, treatment with BM significantly modulated thiobarbituric reactive substances, malonaldehyde, protein carbonyl, glutathione, glutathione-s-transferase and catalase in liver and pancreas of STZ group. The study suggests that BM is effective in reducing hyperglycemia, hyperlipidemia and oxidative stress related to the risk of diabetes. Thus, it may have a therapeutic value for the treatment of T1DM.

Experiment II: Oxidative stress, an imbalance between free radical production and opposing antioxidant defenses is contributed in the pathogenesis of diabetes. The present study was designed to evaluate the beneficial effects of *Terminalia arjuna* (TA) bark extract on streptozotocin (STZ)-induced type 1 diabetes in rats and compared to reference drug, glibenclamide (GL). Diabetes was induced by administration of single low dose of STZ (60 mg/kg body wt, i.p) following free access to 5% glucose water for next 24 hours. TA was given orally at a dose of 500 mg/kg for 4 weeks after diabetes induction. At the end of the experiment, blood was drawn and rats were then sacrificed and their liver and pancreas were dissected for biochemical and histopathological assays. The extract showed significant in vitro antioxidant activity in a dose dependent manner. Levels of fasting blood glucose, glycosylated hemoglobin, total cholesterol, triglycerides, free fatty acids, low density lipoprotein-cholesterol, very low density lipoprotein-cholesterol were increased while the levels of hepatic glycogen, amylase, insulin and high density lipoprotein cholesterol decreased in the STZ-induced diabetic group. These changes were ameliorated by TA supplementation. The STZ group showed elevated renal injury markers in serum, including blood urea nitrogen, serum
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creatinine and alkaline phosphatase, which were decreased significantly by TA treatment. STZ group showed elevated level of nitric oxide, tumor necrosis factor-α and interleukin-1β in serum which were decreased by TA treatment. Moreover, treatment of rats with TA augmented the levels of thiobarbituric reactive substances, malonaldehyde, protein carbonyl and glutathione, and activity of glutathione-s-transferase and catalase in the liver and pancreas of diabetic rats. These results were supported by histopathological examination of the liver and pancreas sections. Our study reveals that TA, as a powerful anti-oxidant, prevents hyperglycemic, hyperlipidemic conditions and oxidative damage in a rat model of type 1 diabetes. Thus, TA is beneficial in the control of diabetes and its related complications.

Experiment III: The present study was designed to evaluate the beneficial effect Pycnogenol® (PYC) on hyperglycemia, lipid profile, renal function markers, inflammatory markers and oxidative damage in diabetic rats and compared to reference drug, glibenclamide (GL). Diabetes was induced in rats by an intraperitoneal injection of streptozotocin (STZ, 60mg/kg body-weight) followed by free access to 5% glucose for the next 24h. Four days after STZ injection, rats were supplemented with PYC (10mg/kg body-weight) for four weeks. At the end of the experiment, blood was drawn and rats were then sacrificed and their livers and pancreases were dissected for biochemical and histological assays. The level of fasting blood glucose and glycosylated hemoglobin significantly increased but amylase, insulin and hepatic glycogen level decreased in STZ group. PYC significantly augmented these effects in STZ + PYC group. The level of total cholesterol, triglycerides, free fatty acids, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol increased while serum insulin and high density lipoprotein cholesterol level decreased significantly in HFD/STZ-induced diabetic rats. These alterations in serum were significantly ameliorated by PYC treatment. The STZ group showed elevated renal injury markers in serum, including blood urea nitrogen, serum creatinine and alkaline phosphatase, which were decreased significantly by TA treatment. STZ group showed elevated level of nitric oxide, tumor necrosis factor-α and interleukin-1β in serum which were decreased by PYC treatment. Moreover, PYC significantly ameliorated increased thiobarbituric reactive substances, malonaldehyde, protein carbonyl, and decreased levels of glutathione, glutathione-s-transferase and catalase activity in the liver and pancreas of the STZ rats. Histopathological and immunohistochemical examination also revealed a remarkable protective effect of PYC. Furthermore, PYC supplementation was also found to increases insulin expression in the pancreas of STZ rats. The study suggests that PYC is effective in reducing diabetic related complications in a type I model of diabetes, and might be beneficial for the treatment of diabetic patients.
STUDY ON TYPE 2 DIABETES

Experiment IV: Recently, diabetic healthcare professionals had a considerable interest in the use of antioxidants, as oxidative stress is known to play an important role in the onset as well as development of further secondary complications of diabetes. Experimental and clinical studies show that naturaceutical antioxidants in diet have antidiabetic potential. This study explores the beneficial effect of *Butea monosperma* (BM) Lam. extract on high fat diet (HFD) and streptozotocin (STZ)-induced diabetes in rats and compared to reference drug, glibenclamide (GL). Diabetes was induced by feeding the rats with HFD for 2 weeks followed by a single injection of STZ (40 mg/kg body weight, intraperitoneally). BM was given orally at a dose of 300 mg/kg for 4 weeks after diabetes induction. At the end of the experiment blood was drawn and rats were then sacrificed, and their liver and pancreas were dissected for biochemical and histopathological studies. The level of fasting blood glucose, glycosylated hemoglobin, blood urea nitrogen, serum creatinine, and alkaline phosphatase, total cholesterol, triglycerides, free fatty acids, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol were significantly increased while hepatic glycogen, insulin and high density lipoprotein cholesterol level decreased in HFD/STZ-induced diabetic rats, which were augmented by BM. HFD/STZ group showed elevated level of nitric oxide, tumor necrosis factor-α and interleukin-1β in serum which were decreased by BM treatment. Furthermore, treatment of rats with BM significantly ameliorated the levels of thiobarbituric reactive substances, malonaldehyde, protein carbonyl and glutathione, and activity of glutathione-s-transferase and catalase in liver and pancreas of diabetic rats. These results were supplemented by histopathological examination in liver and pancreatic sections. Our study reveals that BM, as a powerful antioxidant, prevents diabetic complications and oxidative damage in a rat model of type 2 diabetes. Thus, BM may be implicated as a preventive agent against diabetes mellitus. However, more work is warranted to elucidate its myriad mechanisms of action.

Experiment V: Traditionally, *Terminalia arjuna* (TA) has been used cardiotonic. TA also has been shown to possess antimutagenic, antiulcer, wound healing, antibacterial, hypoglycemic and hypolipidemic properties. The present study was designed to evaluate the beneficial effects of *Terminalia arjuna* (TA) bark extract on hyperglycemia, lipid profile, renal damage markers, inflammatory markers and oxidative stress in the liver and pancreas of rats with type 2 diabetes mellitus (T2DM) and compared to reference drug, glibenclamide (GL). The extract showed significant in vitro antioxidant activity in a dose dependent manner which may be attributed due to presence of high amount of flavonoids, glycosides and saponin. T2DM was induced by feeding rats with a high-fat diet (HFD; 40%) for two weeks followed by single dose of streptozotocin (STZ; 40 mg/kg, intraperitoneally). TA was given
orally at a dose of 500 mg/kg for 4 weeks after diabetes induction, after which blood was drawn. The rats were then sacrificed and their liver and pancreas dissected for biochemical assays. Levels of fasting blood glucose, glycated hemoglobin, total cholesterol, triglycerides, free fatty acid, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol significantly (P<0.05) increased, while serum insulin, hepatic glycogen and high density lipoprotein cholesterol decreased in the HFD/STZ group. TA treatment counteracted these effects in the HFD/STZ + TA group. The HFD/STZ group showed elevated renal injury markers in serum, including blood urea nitrogen, serum creatinine and alkaline phosphatase, which were decreased significantly (P < 0.05) by TA treatment. HFD/STZ group showed elevated level of nitric oxide, tumor necrosis factor-α and interleukin-1β in serum which were decreased by TA treatment. Moreover, treatment with TA significantly (P<0.05) ameliorated thiobarbituric reactive substances, malonaldehyde, protein carbonyl, glutathione, glutathione-s-transferase and catalase in liver and pancreas of the HFD/STZ group. Histopathological examination also revealed partial protective effect of TA in the liver and pancreatic sections of the HFD/STZ rats. These results suggest that TA is effective in reducing hyperglycemia, hyperlipidemia and oxidative stress related to the risk of diabetes. Further studies are needed to determine whether TA could offer alternative treatment for the management of diabetes.

Experiment VI: Abnormal regulation of glucose and impaired carbohydrate utilization that result from a defective or deficient insulin are the key pathogenic events in type 2 diabetes mellitus (T2DM). The present study was design to evaluate the modulating effects of Pycnogenol® (PYC), a naturally occurring antioxidant in normal and diabetic rats and compared to reference drug, glibenclamide (GL). Diabetes was induced by feeding rats with a high-fat diet (HFD; 40%) for 2 weeks followed by an intraperitoneal (IP) injection of streptozotocin (STZ; 40 mg/kg; body weight). An IP dose of 10 mg/kg PYC was given continually for 4 weeks after diabetes induction. At the end of the 4-week period, blood was drawn and the rats were then sacrificed, and their livers and pancreas dissected for biochemical and histopathological assays. In the HFD/STZ group, levels of fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglycerides (TG), free fatty acids (FFAs), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C), significantly increased, while hepatic glycogen level, serum insulin and high density lipoprotein cholesterol (HDL-C) level decreased. PYC supplementation significantly reversed these parameters. The alterations in the level of nitric oxide (NO), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-β) in serum were significantly augmented by PYC treatment. Moreover, supplementation with PYC
significantly ameliorated thiobarbituric reactive substances, malonaldehyde, protein carbonyl, glutathione and antioxidant enzymes [glutathione-s-transferase, catalase] in the liver and pancreas of HFD/STZ rats. These results were supported with histopathological examinations. The study suggests that PYC is effective in reducing hyperglycemia, hyperlipidemia and oxidative stress and may be considered an alternative remedy for the treatment of T2DM and its complications. As interesting results were found with PYC, so we did the further detailed study to evaluate the possible mechanism of action of PYC, using the western blotting and tunnel staining techniques. Treatment with PYC was found to increase the phosphorylation of Akt (pAkt) in the liver of the HFD/STZ rats and to reduce the number of apoptotic β-cell in pancreas tissue of HFD/STZ rats during TUNNEL staining. Although detailed studies are required for the evaluation of the exact protective mechanism of PYC against diabetic complications, these preliminary experimental findings demonstrate that PYC exhibits antidiabetic effects in a rat model of type 2 DM by potentiating the antioxidant defense system. These finding supports the efficacy of PYC for diabetes management.

In conclusion,

- BM augmented FBG, GHb, TC, TG, FFAs, LDL-C and VLDL-C, amylase, insulin, hepatic glycogen and HDL cholesterol levels. It also attenuates renal injury markers including BUN, Scr and ALP and inflammatory markers TNF-α, IL-1β and NO in serum.
- Furthermore, BM modulated increased TBARS, MDA, PC level and decreased GSH, GST, CAT in liver and pancreas.
- Histopathological examination also revealed a remarkable protective effect of BM in the diabetic group.
- It was found to be more effective to metabolic markers in TIDM compared to TA, whereas it was less efficient to normalize renal function markers and lipid profile in both model as compare to TA and PYC.
- TA modulated metabolic markers (FBG, GHb, amylase, insulin, hepatic glycogen) and dyslipidemia markers (TC, TG, FFAs, L-DLC and V-LDLC, H-DLC) efficiently.
- TA augmented renal injury markers (BUN, Scr and ALP) and inflammatory markers (TNF-α, IL-1β and NO) in serum
- Furthermore, TA ameliorated increased oxidative stress markers (TBARS, MDA, PC, GSH, GST, CAT)
Histopathological examination also revealed a remarkable protective effect of TA in the diabetic group.

It significantly attenuated oxidative damage and hyperglycemia but less effective than PYC.

PYC augmented significantly FBG, GHb, TC, TG, FFAs, L-DLC and V-LDL, amylase, insulin, hepatic glycogen and H-DLC levels and also renal injury markers in serum, including BUN, Scr and ALP and inflammatory markers in serum, including TNF-α, IL-1β and NO.

PYC significantly modulated increased TBARS, MDA and PC level and decreased GSH, GST, CAT in the liver and pancreas.

Histopathological findings also revealed a remarkable protective effect of PYC in the diabetic group.

We reported for the first time that PYC has been found to increase the expression of pAKT and insulin. Furthermore PYC reduced TUNEL staining thus preventing apoptosis of pancreatic β-cell.

PYC showed the better results to improve the hyperglycemia as well as normalization of biochemical and histopathological changes than BM and TA.

GL potentially reduced hyperglycemic effects and metabolic markers but found to be less effective in normalizing the diabetic associated abnormalities e.g., antioxidant status, lipid profile when compared to PYC group.

In nutshell, PYC treatment is more effective in reducing diabetic and its related complications in both models of DM compared to BM and TA.

Thus present study reveal that Butea monosperma, Terminalia arjuna and Pycnogenol can be used as anti-hyperglycemic agents for the management of DM. The treatment/prevention of diabetes mellitus (Type I & II) by the above extract might be better and significant than the existing remedies, pending further investigations to trace out the exact mechanistic pathways. It is conclude that PYC must be considered as excellent candidate for future studies on diabetes. Moreover, more emphasis on animal and human studies is needed to confirm the findings of our studies. In addition, further comprehensive pharmacological investigations including experimental chronic studies, need to be carried out.


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