HYPOGLYCEMIC EFFECT OF ACETONE EXTRACT OF TERMINALIA ARJUNA ROXB. BARK ON TYPE-2 DIABETIC ALBINO RATS

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INTRODUCTION
Diabetes mellitus is a chronic metabolic disorder and syndrome characterized by raised glucose level in the blood due to deficiency or diminished effectiveness of insulin with a strong hereditary basis and is usually associated with passage of sugar in the urine. It is also initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Barcelo and Rajpathak, 2001).

Popular interests in alternative medicines and self-prescribed oral nutritional supplements have grown recently (Dham et al., 2006). Although more than 200 pure phyto-chemicals are known to exert anti-hyperglycemic activity. Present anti-diabetic drugs like glimepiride, glipizide, Pioglitazone, Rosiglitazone, Metformin and many more have been used in diabetes therapy. However, they do not cure diabetes. Due to the lack of a complete understanding of how diabetes develops, there is no single cure for diabetes. The pharmacological studies have shown antiviral (Kusumoto et al., 1995) anti-mutagenic (Kaur et al., 2001) antiplague formation (Shaila et al., 1997), anticancer (Avinash et al., 2000) and hypotensive properties (Takahashi et al., 1997) of T. arjuna bark. Terminalia arjuna bark has many therapeutic or medicinal values and is mostly used by rural tribal people for treatment of diarrhea, dysentery, tuberculosis, cough, asthma, earache, cleansing sores, ulcers and syphilitic infection, sex stimulation, skin disorder, relieving excessive menstrual bleeding and leucorrhoea, angina and heart disease in ancient time. T. arjuna leaves plant has shown to be analgesic and anti-inflammatory properties in mice (Moulisha et al., 2011). So, presently effect of T. arjuna bark acetone extract on STZ induced type-2 diabetic albino rats was studied and compared the effects with anti-diabetic drug like glimepiride.

MATERIALS AND METHODS

Animals
Male albino wistar rats (Rattus norvegicus) of 6-8 weeks age (weight approx. 125 gm/rat) were used in this study and housed in stainless steel cages (10 rats/cage). They were acclimatized under laboratory conditions (24 ± 1°C of ambient temperature and relative humidity (55±10%), with a 12:12h light-dark cycle.)
cycle). Animals were fed on standard food and Aqua-guard filtered water ad libitum for whole period of the experiment.

**Grouping of animals**

Sixty animals were distributed into 6 groups (10 rats in each group) as follows- Group-1: normal non-diabetic rats, Group-2: diabetic control rats, Group-3: diabetic rats fed with arjuna bark acetone extract (250 mg/kg body wt.), Group-4: diabetic rats fed with arjuna bark acetone extract (500 mg/kg body wt.), Group-5: diabetic rats fed with Glimepiride (2 mg/kg body wt.) in diet and Group-6: Runner group (normal rats fed with arjuna bark extract @ 500 mg/kg body wt.) for study of bark toxicity. Arjuna bark acetone extract was fed to rats of respective groups for eight weeks continuously. All rats were sacrificed at 8th weeks of experimental period and assessed for various biochemical parameters.

**Preparation of acetone extract of *Terminalia arjuna* bark**

Wet bark of *T. arjuna* plant was collected from Central tasar Research and Training Institute (CTR and TI), Ranchi during September month. *T. arjuna* bark were shade dried for 30 days and one hundred gram of shade dried *T. arjuna* bark was shocked for 48h with 400ml of absolute acetone in an extraction flask. Next day the mixture was filtered with Whatman’s filter paper no-1 and filtrate was collected and dried using vacuum evaporator and residue bark acetone extract were coarsely powdered with the help of mixer grinder.

**Induction of type-2 diabetes in rats**

All animals were acclimatized for two weeks before onset of experiment in laboratory condition. Type-2 Diabetes was induced by feeding of 21% fructose with standard food for four weeks before a single dose of intra-peritoneal injection of 40 mg/kg body weight streptozotocin (Wang et al., 2007). STZ was freshly prepared in 0.1 M citrate phosphate buffer; pH 6.3) all the group of animals except group-1 and VI, those were injected with equal volume of citrate phosphate buffer only. All rats were fasted for 12h before STZ injection in the cage.

**Hypoglycemic studies of *Terminalia arjuna* bark extract**

**Urine sugar test**

The urine sugar were analysed by using commercial urine sugar indicator (Diastix®, Bayer HealthCare, USA) as per manufacturer’s instructions. The test was based on a double sequential enzyme reaction. The results are reported as +1 to +4 depending upon the colour and intensity of the cuprous oxide precipitate.

**Urine Ketone bodies test**

The urine ketone bodies were analysed by using commercial urine ketone bodies indicator (Keto-Diastix®, Bayer HealthCare, USA) as per manufacturer’s instructions.

**Blood Glucose test**

Glucose levels were determined by using one drop of blood samples (drawn from tail vein of rats) in Bayer Contour TS Glucometer (Bayer Healthcare Ltd., Hong Kong) as per manufacturer instructions.

**Oral glucose tolerance test (OGTT)**

OGTT was performed between 0900-1400h on 8th week as per the method described by Tran et al. (2003). The rats were deprived of food for 12-14h before the administration of an oral glucose at a concentration of 2 gm/kg body weight. Blood samples were collected from the tail vein at 0 (before administration), 60 min and 120min after glucose administration. Glucose levels were determined by using one drop of blood samples in Bayer Contour TS-Glucometer (Bayer Healthcare Ltd., Hong Kong).

**Serum urea and creatinine test**

Serum urea and creatinine were determined by commercial kit procured from Crest Biosystems, Goa, India. Serum urea test was based on GLDH Kinetic method and creatinine test was based on Alkaline Picrate method.

**Serum SGPT and SGOT test**

Serum SGPT and SGOT were performed by commercial kit procured from Crest Biosystems, Goa, India. Both tests were based on Reitman and Franklin’s method (Reitman and Franklin’s, 1957).

**Statistical analysis**

The data were analysed by one way ANOVA (Analysis of Variance). Values expressed are Mean ± Standard Error Mean (S.E.M.) of three experiments. Differences in mean were considered significant at (p > 0.05).

**RESULTS**

**Urine sugar and ketone test**

Presence of sugar and ketone bodies in urine of rats of all groups were studied and result are presented in Table 1. Sugar and ketone bodies in urine was found to be higher in rats of control group and was absent in rats of normal groups. Feeding rats with arjuna bark acetone extract (Group-3 and 4) or glimepiride (Group-5) results in decrease in urine sugar and ketone bodies.

**Blood Glucose test**

HFD and single low dose of STZ in rats leads to significant (p > 0.05) increase in blood glucose level. After 24h of STZ injection, in diabetic control group-2, blood glucose level (mg/dL) was significantly (p > 0.05) higher (273.33±7.21) in comparison to rats of normal group-1 (78.00±4.04). After 8th week of experiment, the blood glucose level was found to be 76.66±3.17 in normal rats and 263.66±6.93 in diabetic control rats. Feeding arjuna bark acetone extract (500mg/kg body weight) to STZ treated rats (Group - 4) significantly (p > 0.05) decreased blood glucose level which was non-significant (p > 0.05) to glimepiride treated rats (Group-5). Supplementation of arjuna bark acetone extract (250mg/kg BW, Group-3) to rats decreased blood glucose level (123.33 ± 6.64) which was significantly (p > 0.05) higher than 500mg/kg body weight arjuna bark acetone extract fed rats (84.33 ± 4.63, Group-4). Comparable blood glucose concentration were recorded at 8th weeks of treatment in rats of 500 mg/Kg BW arjuna (84.33 ± 4.63), Glimepiride (80.33 ± 4.63), runner (77.66 ± 3.84) and normal (76.66 ± 3.17) groups (Table 1).

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The data were analysed by one way ANOVA (Analysis of Variance). Values expressed are Mean ± Standard Error Mean (S.E.M.) of three experiments. Differences in mean were considered significant at (p > 0.05).

**RESULTS**
At 8th week of treatment, oral glucose tolerance test was performed in all groups of rats. In all group of rats except control group-2, the blood glucose level came to normal (79.00±6.72) after 120 minutes of glucose administration and was statistically non-significant (p>0.05) with blood glucose level at 0 minute (Fig. 1). In control group-2, blood glucose level (mg/dL) at 120 minutes (273.00±12.52) of administration was significantly (p>0.05) higher than blood glucose level at 0 minute (316.66±7.21).

**Urine sugar and Urine ketone**

The experiment showed that oral glucose tolerance test (OGTT) in 8th week of experiment. In diabetic control group-2, both SGPT and SGOT were found to be significantly (p<0.05) higher (100.51±4.55 U/L and 124.4±2.67 U/L, respectively) in comparison to normal group-1 rats (41.34±2.55 U/L and 62.63±1.29 U/L, respectively). Feeding arjuna bark acetone extract at concentration of 500 mg/kg body weight to STZ treated rats (Group-4) results in significant (p<0.05) decrease in both SGPT and SGOT at 8th week of experiment which was non-significant (p>0.05) to glimepiride treated rats (Group-5). Feeding 250mg/kg body weight of arjuna bark acetone extract to rats (Group-3) decreased both SGPT and SGOT (59.77±1.94 U/L and 79.33±2.78 U/L, respectively) which was significantly (p>0.05) higher than rats (Group-4) fed with 500mg/kg body weight arjuna bark acetone extract (43.55±3.29 U/L and 63.45±3.20 U/L, respectively).

**DISCUSSION**

Diabetes is a global disease with a huge adverse impact on the health and mortality. Traditional plant medicines are used throughout the world for the treatment of diabetes mellitus. Diabetes mellitus is the world’s largest growing metabolic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels (Tanko et al., 2008).

The present study revealed that acetone extract of *Terminalia arjuna* bark has good effect in lowering blood glucose level in STZ induced type-2 diabetic rats (Table 1). Glimepiride showed maximum reduction of blood glucose level in diabetic rats and at the same time maximum reduction was obtained from arjuna bark extract at a dose of 500mg/kg body weight. STZ induced type-2 diabetic animal showed a significant (p<0.05) increase in blood and urine glucose level, urine ketone bodies, serum urea, creatinine, SGPT and SGOT levels as compared to normal group animals. The increase in serum urea and creatinine levels may be due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume. Several studies also have shown increased correlation between serum urea and creatinine in diabetic patients (Mogenson et al., 1985). Chronic treatment of *T. arjuna* bark to diabetic rats decreased blood glucose level and increase insulin level in STZ diabetic rats. These effects may be attributed to either inhibition of increase in insulin input, inhibition of the intestinal absorption of glucose and increase in glucose metabolism. The experiment showed that oral glucose tolerance test (OGTT) measures the body ability to use glucose, the body’s main source of energy (Gold, 1970). This test can be used to
diagnose pre-diabetes and diabetes. Glucose lowering effects were found after oral administration of acetone extracts in rats (Fig. 1). This may be due to the presence of hypoglycemic flavonoids (Voilley et al., 2004) and tannins (Gupta et al., 2004). The extracts may have the properties to stimulate or regenerate the β-cell for the secretion of insulin and are most effective for controlling diabetes by various mechanisms which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level (Ali et al., 2012) Induction of diabetes with STZ was associated with decrease in hepatic glycogen, which could be attributed to decrease in the availability of the active form of enzyme glycogen synthetase probably because of low levels of insulin (Goel et al., 2004). Decreased activities of the enzymes involved in glucose homeostasis resulting in depletion of liver and muscle glycogen content (Brown et al., 1998). Treatment with plant extracts might increase the level of enzyme to the control level indicating an over-all increase in glucose influx.

In the present study, blood glucose level, OGTT, Kidney and Liver function tests in serum was found to be significantly (p<0.05) low in rats fed with 500mg/kg body weight arjuna bark extract as compared to 250mg/kg body weight arjuna bark extract when fed for 8 weeks.

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REFERENCES


