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

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ABSTRACT
In the present study, hypoglycemic effects of *Terminalia arjuna* bark extract were seen in high fructose (21%) followed by streptozotocin (40mg/kg BW) induced type-2 diabetic male albino rats. *In vivo* study showed protective effect of *T. arjuna* bark acetone extract of towards blood glucose, serum urea, serum createnine, SGOT, SGPT, oral glucose tolerance (OGTT), urine sugar and urine ketone bodies in diabetic rats. Feeding 500 mg/kg BW arjuna bark extract to rats showed better effect for blood and urine parameters as compared to rats fed with 250 mg/kg BW arjuna bark extract. The effect of feeding 500 mg/kg BW arjuna bark extract was found to be almost equal to that of with glimepiride fed diabetic rats. The result indicated that *Terminalia arjuna* bark acetone extract of have antidiabetogenic and possess hypoglycemic effects in type-2 diabetic rats.

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 and metformin not cure diabetes and posses its side effects leads to other complications. So, herbal drugs are gaining popularity in the treatment of diabetes mellitus. The major advances of herbal medicine seem to be their efficacy, low incidence of side effects and low cost. Several plant extract have been shown in different animal models to possess beneficial effect for treatment of diabetes mellitus. *Terminalia arjuna* belongs to Combretaceae family. It is a large size deciduous evergreen plant found in plenty throughout Indo sub Himalayan tracts of Uttar Pradesh, Jharkhand, South Bihar, Madhya Pradesh, Delhi, Deccan region.

Terminalia arjuna bark extract contains acids (arjunic acid, arjunolic acid, arjungenin acid and arjunglycesides and terminic acid), glycosides (argentine arjunosides I-IV), strong antioxidants (flavonoids, tannins, oligomeric proanthocyanidins) and minerals. 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, tubercular caugh, asthama, earache, cleansing sores, ulcers and syphilitic infection, sex stimulation, skin disorder, relieving excessive menstrual bleeding and leucorrhea, 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 $\pm$1ºC of ambient temperature and relative humidity (55$\pm$10%), with a 12:12h light-dark

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 a 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- I 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 Frankel's method (Reitman and Frankel'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 ($p > 0.05$) high (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).

Oral glucose tolerance test (OGTT)

Table 1: Effect of acetone extract of *Terminalia arjuna* bark on various biochemical parameters indicating its anti-hyperglycemic effects after 8th weeks of experiment

Parameters	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6
Urine sugar	-ve	+ 4	+ 1	-ve	-ve	-ve
Urine ketone	-ve	Trace	Trace	-ve	-ve	-ve
Blood glucose* (mg/dL)	76.66 ± 3.17	263.66 ± 6.93	123.33 ± 6.64	84.33 ± 4.63	80.33 ± 4.63	77.66 ± 3.84
Serum urea* (mg/dL)	27.79 ± 1.21	76.14 ± 2.09	37.72 ± 2.59	28.94 ± 1.97	29.38 ± 1.45	27.94 ± 1.57
Se. Creatinine* (mg/dL)	0.80 ± .0.04	1.71 ± 0.06	1.01 ± 0.07	0.83 ± 0.01	0.81 ± 0.01	0.79 ± 0.03
SGPT* (U/L)	40.82 ± 1.56	101.28 ± 5.04	80.44 ± 3.33	62.73 ± 3.42	61.04 ± 4.07	41.30 ± 3.07
SGOT* (U/L)	63.42 ± 1.82	126.53 ± 3.79	101.33 ± 2.17	84.70 ± 2.13	86.34 ± 2.38	63.66 ± 2.15

*significant at $p < 0.05$.

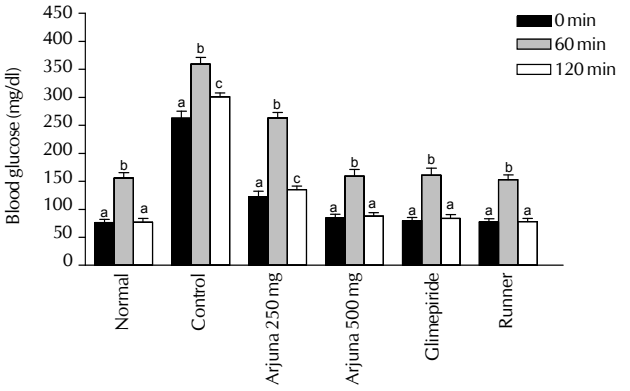

Figure 1: Performance of all groups of rats for Oral Glucose Tolerance Test (OGTT) on 8th week of experiments. (Values are Mean ± S.E.M. of three experiments, Means with different letters are significantly differ at ($p < 0.05$)

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).

Serum urea and creatinine test

Streptozotocin treatment in rats leads to significant ($p > 0.05$) increase in both serum urea and creatinine level as seen in 8th week of experiment. In diabetic control group-2, both serum urea and creatinine were significantly ($p > 0.05$) high (75.85 ± 2.60 mg/dL and 1.714 ± 0.061 mg/dL, respectively) in comparison to normal group-1 rats (27.79 ± 1.21mg/dL and 0.806 ± 0.042 mg/dL, respectively). Feeding of 500mg/kg body weight of arjuna bark acetone extract to STZ treated rats (Group-4) results in significant ($p > 0.05$) decrease in both serum urea and creatinine at 8th week of experiment which was non-significant ($p > 0.05$) to glimepiride treated rats (29.38 ± 1.45, 0.814 ± 0.01 respectively). Supplementation of 250mg/kg body weight of arjuna bark acetone extract to rats (Group-3) results in decrease serum urea and creatinine (37.72 ± 2.59mg/dL and 1.01 ± 0.07mg/dL respectively) which was significantly ($p > 0.05$) higher than rats (Group-4) fed with arjuna bark acetone extract at concentration of 500mg/kg body weight (28.94 ± 1.97 mg/dL and 0.83 ± 0.01 mg/dL respectively).

SGPT and SGOT test

Diabetes induced by streptozotocin to rats leads to significant ($p > 0.05$) increase in both SGPT and SGOT level as seen 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 ß-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 in liver and kidney such as hexokinase has been reported in diabetic animal 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

Ali, M. A., Sultana, M. C., Rahman, B. M., Khatune, N. A. and Wahed, M. I. I. 2012. Antidiabetic activity of ethanolic extract of *Semecarpus anacardium* (linn.) stem barks in normal and alloxan induced diabetic rats. *IJPSR*. **3(8):** 2680-2685.

Augusti, K. T., Anuradha, S. P., Prabha, K. B., Smitha, M., Sudheesh, A. and George, M. C. 2005. Nutraceutical effects of garlic oil, its nonpolar fraction and a ficus flavonoid as compared to vitamin E in CCL4 induced liver damage in rats. *Indian J. Exp. Biol.* **43(5):** 437-444.

Avinash, N., Laxman, S. M., Satwinderject, K., Iqbal, S. G., Renu, W. and Sunil, C. K. 2000. Growth suppression of human transformed cells by treatment with bark extracts from a medicinal plant *Terminalia arjuna*, in vitro cell. *Dev. Biol-Animal*. **36:** 544-547.

Barcelo, A. and Rajpathak, S. 2001. Incidence and prevalence of diabetes mellitus in the Americas. *American J. Public Health*. **10:** 300-308.

Benzie, F. F. and Strain, J. J. 1999. Ferric Reducing/ Antioxidant Power Assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*. **299:** 15-23.

Brown, J. E. and Evans, C. A. R. 1998. Luteolin rich artichoke extract protects low density lipoproti from oxidation in vitro. *Free Radic. Res.* **29:** 242-255.

Dham, S., Shah, V., Hirsch, S. and Banerji, M. A. 2006. The role of complementary and alternative medicine in diabetes. *Curr. Diab. Rep.* **6(3):** 251-258.

Goel, R. K., Mahajan, M. P. and Kulkarni, S. K. 2004. Evaluation of anti-hyperglycemic activity of some novel monocyclic beta lactams. *J. Pharm. Sci.* **7(1):** 80-83.

Gold, A. H. 1970. The effect of diabetes and insulin on liver glycogen synthetase activation. *J. Biol. Chem.* **245:** 903-905.

Gregori, D., Pagano, E., Rosato, R., Bo, S., Zigon, G. and Merletti, F. 2006. Evaluating hospital costs in type 2 diabetes care: does the choice of the model matter? *Curr. Med. Res. Opin.* **22(10):** 1965-1971.

Kaur, S., Grover, I. S. and Kumar, S. 2001. Anti mutagenic potential of extracts isolated from *Terminalia arjuna*. *J. Environ Pathol Toxicol Oncol.* **20:** 9-14.

Kusumoto, I. T., Nakabayashi, T., Kida, H., Miyashrio Hattori, M., Namba, T. and Shimotohno 1995. Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus-I (HIVI) protease. *Phytotherapy Research*. **9(3):** 180.

Mogenson, C. E. and Christensen, C. K. 1985. Blood pressure changes and renal function in incipient and overt diabetic nephropathy. *Hypertention*. **7(6 Pt 2):** 1164-1173.

Moulisha, B., Kaushik, B., Tarun, K. K., Sanjib, B., Ashoke, K. G. and Pallab, K. H. 2011. Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf. *J. Phytology*. **3(1):** 33-38.

Mythilli, P., Parameswari, C. S. and Dayana, J. 2012. Phytochemical analysis of the bark extract of *Terminalia arjuna* and its cardioprotective effect. *Indian J. Innovations and Developments*. **1(8):** 40-42.

Reitman, S. and Frankel, S. 1957. Determination method of SGPT and SGOT activity in serum. *Amer J. Clin. Path*. **28:** 56.

Robert, J. H., Angela, K. Y. and Sean, M. L.. 2003. Vitamin C Inhibits Lipid Oxidation in Human HDL. *J. Nutr.*, **133(10):** 3047-3051.

Shaila, H. P., Udupa, S. L., Udupa, A. L and Nair, N. S. 1997. Effect of *T. arjuna* on experimental hyperlipidemia in rabbits. *J. Pharmacogn.* **35:** 1-4.

Takahashi, S., Tanaka, H., Hano, V., Ito, K., Nomura, T. and Shigenobu, K. 1997. Hypotensive effect in rats of hydrophilic extract from *Terminalia arjuna* containing tannin, related compounds. *Phytotherapy Res.*. **11(6):** 424- 427.

Tanko, Y. 2008. Hypoglycemic Activity of Methanolic Stem Bark of Adansonnia digitata extract on blood glucose levels of streptozotocin-induced diabetic wistar rats. *International J. Applied Research in Natural Products*. **1(2):** 32-36.

Tran, T. T., Gupta, N., Goh, T., Naigamwalla, D., Chia, M. C., Koohestani, N., Mehrotra, S., McKeown-Eyssen, G., Giacca, A. and Bruce, W. R. 2003. Direct measure of insulin sensitivity with the hyperinsulinemic-euglycemic clamp and surrogate measures of insulin sensitivity with the oral glucose tolerance test: correlations with aberrant crypt foci promotion in rats. *Cancer Epidemiol. Biomarkers Prev.* **12(1):** 47-56.

Viswanatha Swamy, A. H. M., Wangikar, U., Koti, B. C., Thippeswamy, A. H. M, Ronad, P.M and Manjulandian, D. V. 2011. Cardioprotective effect of ascorbic acid on doxorubicin-induced myocardial toxicity in rats. *J. Pharmacol.* **43(5):** 507–511.

Voilley, N. 2004. Acids-Sensing Ion channels (ASICs): New target for the analgesic effects of Non Steroidal Anti-Inflammatory Drugs (NSAIDs). *Curr. Drug Targets Inflam. Allerg.* **3:** 71-79.

Wang, H. J., Jin, Y. X., Shen, W., Neng, J., Wu, T., Li, Y. J. and Fu Z. W. 2007. Low dose Streptozotocin (STZ) combined with high energy intake can effectively induce type-2 diabetes through altering the related gene expression. *Asian Pac J. Clin Nutr.* **16(1):** 412-217.