ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 2 Issue 1

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

Phytochemical properties, total antioxidant status of acetone and methanol extract of *Terminalia arjuna* Roxb. bark and its hypoglycemic effect on Type-II diabetic albino rats.

Chandan Kumar[1*], Raj Kumar[2], Shamshun Nehar[1]

1. Department of Zoology, Ranchi University, Ranchi, India
2. Division of Animal Biochemistry, National Dairy Research Institute, Karnal, Haryana, India
[E-mail: chandanctri@gmail.com; Tel: +91-7209792982]

In the present study, acetone and methanol extract of *T. arjuna* Roxb. bark was evaluated for its phytochemical properties and antioxidant status by FRAP method. The result indicated that tannin, alkaloid, triterpenoid, flavonoid, phytosteroids and saponin are present in both the acetone and methanol extract. Total antioxidant status was found to be significantly ($P<0.01$) higher in acetone extract (212.5±11.55 µM) as compared to methanol extract (35.50±4.70 µM). This indicates higher antioxidative property of *T. arjuna* bark acetone extract in comparison to methanol extract and selected for *in vivo* study. *In vivo* study, showed protective effect of acetone extract of *T. arjuna* bark towards blood glucose, oral glucose tolerance (OGTT) and insulin tolarence test in STZ treated rats. Feeding 500 mg/kg bw arjuna bark extract to rats showed significantly ($P<0.05$) lower parameters as compared to rats of diabetic control rats and 250 mg/kg bw arjuna bark fed rats and was non-significant with glimepiride fed rats. Feeding 500 mg/kg bw extract showed significantly ($P<0.05$) higher anti-hyperglycemic and anti-diabetic effects as compared to 250 mg/kg bw extract. It is concluded that the acetone extract of *Terminalia arjuna* bark exhibit antioxidant power and hypoglycemic properties.

Keyword: *Terminalia arjuna* bark, FRAP assay, Phytochemical properties, Blood glucose, OGTT, Insulin tolerance, Hypoglycemia.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder and is also a syndrome identified by a raised glucose level in the blood (hyperglycemia) due to deficiency or diminished effectiveness of insulin with a strong hereditary basis. It is usually associated with passage of sugar in the urine and 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[1]. There are two major forms of diabetes, type-I (consequence of destruction of pancreatic β-cells, which leads to insulin deficiency) and type-II diabetes (characterized by insulin resistance and relative, rather than absolute, insulin deficiency) which contribute about 10% and 90% of diabetic population, respectively. Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia resulting from defects in insulin secretion or insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart, and blood vessels. In the last few years there has been

an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. India is the largest producer of medicinal herbs and is called as Botanical garden of the world[2]. Although several therapies are in use for treatment of diabetes, there are certain limitations due to high cost and side effects such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity[3].

Terminalia arjuna Roxb. bark is one such ayurvedic remedy that has been mentioned in many ancient Indian medicinal literature including Charaka Samhita and Astang Hridayam, to possess cardio protective property. It is an important cardiotonic plant described in the Ayurveda[4]. It is commonly known as Arjun plant in Hindi language. *Terminalia arjuna* is a large, evergreen deciduous tree found throughout India in plenty throughout Indo sub Himalayan tracts of Uttar Pradesh, Jharkhand, South Bihar, Madhya Pradesh, Delhi, Deccan region mainly along riverside, riverlets and ponds and belongs to *Combrataceae* family. The tree is about 60-80 feet height often buttressed trunk and horizontally spreading branches. The bark is smooth in texture. Leaves are sub opposite, oblong or elliptic usually 4-6 inch in length, suddenly narrowed at the base, often cordate, obtuse or very shortly acute at the apex. The upper part of the leaf is light green in color and lower part is dark in color. Flowers are small, sessile with panicled spikes and occur all round white or yellow stalk. Flowering stage of arjuna is in summer whereas fruiting stage is in winter. Fruits are ovoid, fibrous woody, 2.3-3.5 cm long, globrous with 5-7 hard wings, straighted with numerous curved veins. Arjuna seeds are hard germinating, usually takes 50-76 days. It is generally cultivated on variety of soils but prefers fertile alluvial loam and deep sandy well drained soil[5].

Table-1: Qualitative Phytochemical Properties of Acetone and Methanol Extract of *Terminalia arjuna* Bark Extract. (Values are Mean± S.E.M. of three Experiments, Means with Different Letters are Significantly differ at ($P<0.01$).

Parameters	*T. arjuna* acetone extract	*T. arjuna* methanol extract
Antioxidant power (µM)	212.5±11.55^{a}	35.50±4.70^{b}
Tannins	Present	Present
Triterpenoids	Present	Present
Alkaloids	Present	Present
Flavonoids	Present	Present
Phytosteroids	Present	Present
Saponin	Present	Present

The bark *T. arjuna* has many therapeutic or medicinal values from ancient time 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. The leaves of this plant have analgesic and anti-inflamatory properties studied in mice[6]. The 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), minerals etc. A number of experimental and clinical studies have proved that dried bark powder of this plant have potent hypolipidemic and cardioprotective activity. The pharmacological studies have shown antibacterial, antiviral, anti mutagenic, antiplague, anticancer, antiacne and hypotensive properties of the bark of *T. arjuna*. The use of *Terminalia arjuna* bark in the management of

hypercholesterolaemia has been widely reported due to its high antioxidative properties.

The present study was thus taken up with the objective of understanding the phytochemical properties and antioxidant status of *Terminalia arjuna* Roxb. Bark for the study of antihyperglycemic activity of *T. arjuna* bark on Type-II diabetic albino rats.

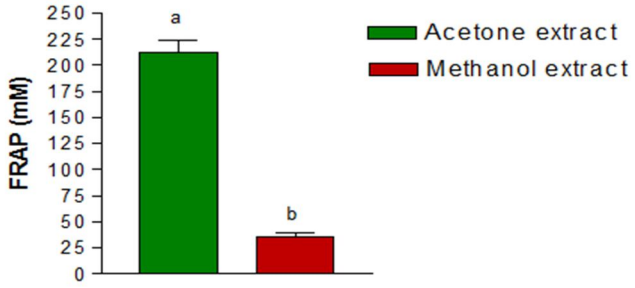

Fig 1: Quantitative Comparison Study of Total Antioxidant Status of Acetone and Methanol Extract of *Terminalia arjuna* bark Extract by FRAP Assay (Values are Mean± S.E.M. of three Experiments, Means with Different Letters are Significantly Different at (*P<0.01*).

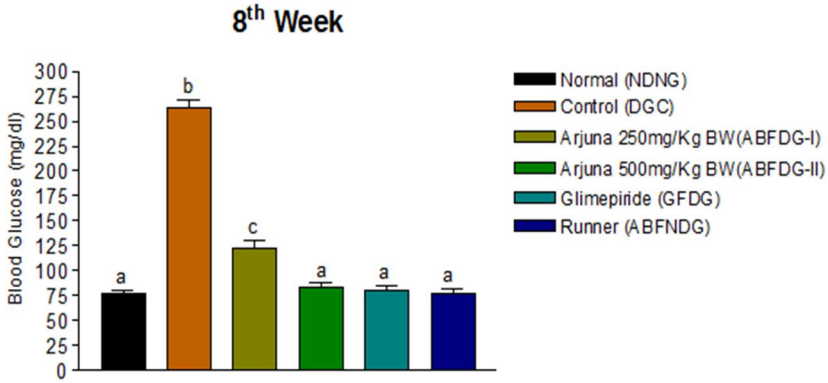

Fig 2: Effect of Acetone Extract of *Terminalia arjuna* bark on Blood Glucose Level at the 8th week of Treatment Indicating its Anti-Hyperglycemic Effects. (Values are Mean± S.E.M. of three experiments, Means with Different Letters are Significantly Different at (*P<0.05*).

2. Material and Methods

2.1 Animals:

Only male albino wistar rats (*Rattus norvegicus)* of weight approx. 125gm/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 12:12 h light-dark cycle). Animals were fed on standard chow and Aqua-guard filtered water *ad libitum* for whole period of the experiment (8 weeks).

2.2 Grouping of animals:

Sixty animals were distributed into 6 groups (ten rats in each group) as follows-

Group-I: normal non-diabetic rats,
Group-II: diabetic control rats.
Group-III: diabetic rats fed with arjuna bark acetone extract (250 mg/kg body wt.).
Group-IV: diabetic rats fed with arjuna bark acetone extract (500 mg/kg body wt.),
Group-V: diabetic rats fed with Glimepiride (2 mg/kg body wt.) in diet.
Group-VI: Runner group (normal rats fed with arjuna bark extract @ 500 mg/kg body wt.) for study of bark toxicity.

All rats were sacrificed at 8th weeks of experimental period and assessed for various biochemical parameters.

2.3 Induction of Type-II Diabetes in Rats:

All animals were acclimatized for two weeks before onset of experiment in laboratory. Type-II Diabetes was induced to all animals except rats of group-I and VI by feeding 21% fructose with standard chow for four weeks before a single dose of intra-peritoneal injection of 40 mg/kg body weight streptozotocin[7], those were fasted for 12 hours before STZ injection. STZ was freshly prepared in 0.1M citrate phosphate buffer (pH 6.3).

2.4 Composition of Standard Chow:

All rats were fed with standard chow containing Wheat (259gm/kg), Bengal gram (463gm/kg), Groundnut (195gm/kg), Refined oil (67gm/kg), Vitamin mixture (01gm/kg) and Mineral mixture (15gm/kg). Total nutrient value of standard chow was calculated as Carbohydrate (59.47%), Protein (16.64%) and Fat (6.39%) determined by Kjeldahl method[8].

2.5 Collection of *Terminalia arjuna* Bark

The *Terminalia arjuna* Roxb. plant specimen was identified by K. Karthigeyan, Scientist-'C', Central National Herbarium, Botanical Survey of India, Howrah, Kolkata via memo no.- CNH/8/2012/Tech.II/680. After that wet bark of *Terminalia arjuna* plant was collected from Tasar silkworm (*Antheraea mylitta* D.) food plant farm of 'Central Tasar Research & Training Institute (CTR&TI), Nagri, Ranchi' during September month.

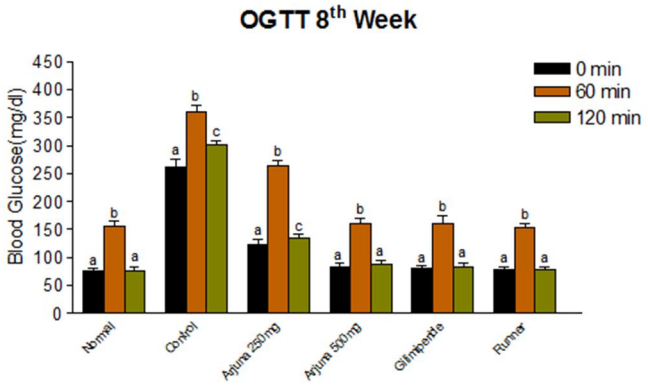

Fig 3: Effect of Acetone Extract of *Terminalia arjuna* bark and Performance of all Groups of rats for Oral Glucose Tolerance Test (OGTT) on 8th week of Experiment. (Values are Mean± S.E.M. of three Experiments, Means with Different Letters are Significantly Different at ($P<0.05$).

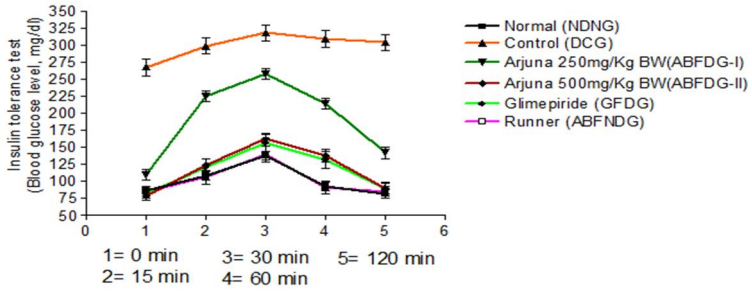

Fig 4: Effect of Acetone Extract of *Terminalia arjuna* Bark on Insulin Tolerance Test at Last Day (8th Week of Treatment) (Values are Mean± S.E.M. and Significantly Different at ($P<0.05$).

2.6 Preparation of Acetone and Methanol Extract of *Terminalia arjuna* Bark:

Terminalia arjuna bark were shade dried for 45 days and powdered using mixer grinder. One hundred gram of shade dried *T. arjuna* bark powder was soaked for 48 hours in extraction flask containing 400 ml of absolute acetone (or methanol for methanol extract). The flask was kept at room temperature with continuous mixing in every 2 hours. Next day the mixture was filtered with whatman's filter paper no-1 and filtrate was collected. Unfiltered plant material was again soaked in equal volume of acetone for two times. All the filtrate were pooled and dried using vacuum evaporator and residue bark acetone extract were coarsely powdered with the help of mixer grinder. Both acetone and methanol extract were stored separately at 4^0C in refrigerator for further use.

2.7 Phytochemical Analysis of Acetone and Methanol Extract:

Phytochemical tests were performed qualitatively using cold acetone and metanol extract of *T. arjuna* bark according to following methods:

a) **Ferric Chloride Test for Tannin:** 500mg of extract was mixed with 10 ml of distilled water in a test tube and then filtered. Few drops of 1% ferric chloride solution was added to 2ml of the filtrate. Occurrence of a blue-black precipitate indicates the presence of tannins.

b) **Test for Triterpenoid:** 10 mg of the extract was mixed with 1 ml of chloroform in a test tube and then 1 ml of acetic anhydride was added and warmed. After cooling, 2 ml of conc. H_2SO_4 was added to it. Formation of reddish violet colour indicates the presence of triterpenoid.

c) **Mayer's test for Alkaloid:** 1ml of extract was added in 2 ml of Mayer's reagent in a test tube. Mayer's reagent was freshly prepared by dissolving a mixture of mercuric chloride (1.36g) and of potassium iodide (5g) in water (100ml). Cream colored precipitate confirms the presence of alkaloid in test sample.

d) **Shinoda Test for Flavonoid:** 5 drops of diluted HCl was added in 0.5 ml of bark extract and then a small piece of magnesium was added on it in a test tube. Appearance of reddish pink colouration indicates the presence of flavonoid in test sample.

e) **Salkowski's Test for Phytosteroid:** 1ml of extract was treated with chloroform and a drop of concentrated sulfuric acid was added to the solution in a test tube. Formation of reddish brown color indicates the presence of phytosteroid.

f) **Foaming test for Saponin:** 300mg of extract was boiled with 5 ml of distilled water in a test tube and filtered. About 3ml of distilled water was further added to the filtrate and shaken vigorously for about 5 minutes. Frothing which persist on warming indicates the presence of saponin[9].

2.8 Total Antioxidant Status of Acetone and Methanol Extract (FRAP Method):

Total antioxidant status was measured using ferric reducing antioxidant power (FRAP) assay[10]. FRAP reagent was prepared by mixing 300mM acetate buffer, pH 3.6, 10mM 2,4,6-tripyridyl-S-triazine (TPTZ) in 40mM HCl and 20mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1. Aqueous solution of freshly prepared pure antioxidant solution such as ascorbic acid (1000µM) was used as standard. Acetone and Methanol extract were made 1g/ml in acetate buffer and its 100 µl was mixed with 3ml of working FRAP reagent separately (for test solution). Then it was vortex mixed and absorbance at 593nm was measured at 0 min and at 5 min. against a reagent blank (FRAP solution). The change in absorbance was translated into FRAP value (µM) by relating A_{593nm} of test sample with that of standard solution (ascorbic acid 100µl+FRAP solution 3ml) of known FRAP value using formula:

$$\frac{\text{0–5 min } \Delta A_{593} \text{ test sample}}{\text{0–5 min } \Delta A_{593} \text{ standard}} \times \text{FRAP value of standard}$$

FRAP value of 1000 µM ascorbic acid is 2000, which was taken as standard.

2.9 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.

2.10 Oral Glucose Tolerance Test:
OGTT was performed between 0900-1400 h on 8th week as per the method described by Tran *et. al.*, 2003[11]. The rats were deprived of food for 12-14 h before the administration of an oral glucose @ 2 g/kg body weight (200g/l solution). Blood samples were collected from the tail vein at 0 (before administration), 60 min and 120 min after glucose administration. Glucose levels were determined by using one drop of blood samples in Bayer Contour TS Glucometer (Bayer Healthcare Ltd., Hong Kong).

2.11 Insulin Tolerance Test:
After an overnight fast, rats were orally ingested with 2 g/ kg body weight of glucose solution and simultaneously injected (I.V.) with 2 U/ kg body weight human regular insulin (M.J. Biopharm Pvt. Ltd., Rajgad, India). Blood samples were taken at various time intervals i.e. 0, 15, 30, 60 and 120 min, and blood glucose concentrations were measured by using one drop of blood samples in Bayer Contour TS Glucometer (Bayer Healthcare Ltd., Hong Kong).

3. Result
3.1 Phytochemical Analysis:
The qualitative assessment of *T. arjuna* bark extract indicates that tannin, alkaloid, triterpenoid, flavonoid, phytosteroids and saponin are present in both the acetone and methanol extract (Table-1).

3.2 Total Antioxidant FRAP Test:
Acetone and methanol extract of *T. arjuna* bark was compared for its total antioxidant status by FRAP assay. Total antioxidant status was found to be significantly ($P<0.01$) higher in acetone extract (212.5±11.55 µM) as compared to methanol extract (35.50±4.70 µM). This indicates higher antioxidative property of *T. arjuna* bark acetone extract in comparison to methanol extract (Table-1 and figure-1).

3.3 Blood glucose test:
Blood glucose level was found to be 78.00±4.04 and 273.33±7.21 respectively in rats of normal and diabetic control group (figure-2). Feeding 500 mg/Kg BW arjuna bark (group-IV) acetone extract to diabetic rats showed significantly ($P<0.05$) lower (124.33±6.64) blood glucose as compared to diabetic control rats (273.33±7.21) and rats fed with 250 mg/Kg BW arjuna bark (group-III) extract (184.66±9.82). Blood glucose in arjuna bark fed rats were comparable with glimepiride (122.33±6.93) fed rats but it was significantly ($P<0.05$) higher than rats of normal (78.00±4.04) and runner group (78.66±3.75).

3.4 Oral Glucose Tolerance Test:
At the 8th weeks of treatment (Figure-3), blood glucose concentration was restored back to normal (79.00±6.72) in rats of 500 mg/Kg BW arjuna (group-IV) or glimepiride (group-V) group. Rats fed with 500 mg/Kg BW arjuna bark acetone extract showed significantly ($P<0.05$) better oral glucose tolerance as compared to rats fed with 250 mg/Kg BW arjuna bark extract. Effects of arjuna bark at the concentration of 500 mg/Kg BW was found to be comparable with glimepiride fed (group-V) and rats of normal (group-I) and runner group (group-VI). In diabetic control group-II rats, 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).

3.5 Insulin Tolerance Test:
An insulin tolerance test is a diagnostic procedure to assess pituitary function. Rats of diabetic control group (group-II) showed resistance towards insulin treatment. A non-significant ($P>0.05$) decrease in blood glucose (mg/dl) was observed in rats of diabetic control group (group-II) at 30 min (318±11.81) and 90 min (304±11.46) of insulin administration (figure-4). Rats of non-diabetic normal (group-I, 138±5.61 vs. 82±5.76) and runner (group-VI, 140±11.11

vs. 85±7.16) groups showed statistically significant (*P<0.05*) decrease in blood glucose at 30 min and 60 min. Feeding arjuna acetone extract bark to diabetic rat group improved the insulin tolerance and showed blood glucose back to normal. Arjuna bark acetone extract at the concentration of 500 mg/Kg BW (162±9.33 Vs 90±8.36) and glimepiride (156±12.73 Vs 89±7.76) showed significantly better insulin tolerance at 30 min and 60 min as compared to rats fed with that of 250 mg/Kg BW (258±8.11 Vs 142±8.26).

4. Discussion

The uses of traditional medicines as an alternative therapy for the treatment of diabetes have been evolved in certain ethnic groups with a high prevalence of disease among Native Americans, Hispanics, and Asians[12]. The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Different species of medicinal plants are used in the treatment of diabetes mellitus. For diabetes treatment, before the discovery of insulin by Banting and Best in 1922[13], the only options were those based on traditional practices[14]. In the present study, tannin, alkaloid, flavonoid, trierpenoid, phytosteroids and saponin were reported in both the acetone and methanol extract of *T. arjuna* bark. In the similar line of study, Mythili P. *et. al.*, 2012[15] have found phytosterols (b-sitosterol), flavonoids (arjunone, arjunolone, and luteolin), terpenes, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides) in *Terminalia arjuna* bark triterpenoids extract. Flavonoids (Arjunic acid, arjunolic acid, arjungenin acid and arjunglycesides) being powerful antioxidants are reported to play a role in antihyperglycemic and analgesic activity by targeting pancreas and prostaglandins since stem bark of *T. arjuna* plant contain flavonoids and glycosides. The phytochemical screening of the plant *T. arjuna* stem bark showed the presence of flavonoids, alkaloid, triterpenoids, saponins and tannins shown in Table-1. Amino acid and resins are absent in bark of *T. arjuna*. Triterpenoids, flavonoids, tannins and phytosteroids possess significant (*P<0.05*) antioxidant antihyperglycemic[16] and cardioprotective effect[17]. Triterpenoids have same effect as vitamin-C. Saponin compound may be responsible for intropic effects of *T. arjuna*. Arjunic acid maintained the intracellular concentration of Ca^{2+} and Ca^{2+} homeostasis; in turn regulates the Ca^{2+} involved single transduction pathways[15]. Triterpenoids extract of *T. arjuna* have ability to prevent myocardial abnormalities and pathological changes in biochemical marker, which are induced by cyclosporine A.

In a study, Singh *et. al.*, 2012[18] found higher antioxidant power of acetone extract *T. chebula* fruits which were followed by methanol extract. They reported antioxidant power at low concentrations (6×10^{-4} g/l) of ethyl acetate and acetone extracts were possess highest scavenging power as compared to methanolic and aqueous extracts. In the present study, we have found similar type of result in which total antioxidant power of acetone extract (212.50±11.55) of *T. arjuna* bark were found to be significantly (*P<0.05*) higher as compared to methanol extract (35.50±4.70). In a recent study, Amira *et.al.*, 2013[19] found comparable antioxidant power of acetone and methanol extract of bitter melon (*Momordica charantia*) as measured by FRAP method. The present investigation established that the stem bark of the plant *T. arjuna* have bioactive principles with anti-diabetic potential (figure-1).

High fructose diet followed by STZ injection constantly increased in blood glucose in rats which were came to normal after feeding *T. arjuna* bark acetone extract (500 mg/kg BW) for 8 weeks, in the present studies (figure-2). Similar results were obtained by Wilson and Islam, 2012[20] showed increased blood sugar in normal rats after HF-STZ treatment. Ragavan and Krishnakumari, 2006[16] showed 500mg/kg BW oral administration of ethanol extract of *T. arjuna* bark for 30 days results in significant (*P<0.05*) reduction in blood glucose (mg/dl) from 302.67±22.35 to 113.17 ± 14.25. Similarly, Nagappa *et.al.*, 2003[21] reported that

administration of alloxan (150 mg/kg, i.p.) to wistar albino rats led to 1.5-fold elevation of fasting blood glucose levels. Workers has given daily treatment of various extract of *Terminalia catappa* for three weeks and found a dose-dependent fall in blood sugar levels by 25–62%.

Rats of type-II diabetic as compared to normal group showed lower tolerance towards oral administration of glucose and higher blood glucose even after 120 min of glucose administration in OGTT (figure-3). Feeding 500 mg/kg BW *T. arjuna* bark acetone extract or gilimeperide to diabetic rats for 8 weeks restored the OGT potential. In a similar line of study, Murali *et.al.*, 2004[22] fed water extract of dry fruits of *Terminalia chebula* to STZ induced diabetic rats at a dose of 200 mg/kg BW for 2 weeks and found improved glucose tolerance as indicated by 44% of reduction in the peak blood glucose at 2nd hour in glucose tolerance test. In the present study diabetic rats fed with 500 mg/kg BW acetone extract of *T. arjuna* bark or glimepiride, for 8 weeks, improved glucose utilization during GTT (44.3% reduction in blood glucose at 2hrs) within 90 min after administration of the extract. Recently, Govinda *et.al.* (2012[23] reported improved OGTT of STZ induced diabetic male albino wistar rats after oral administration of hydro-alcoholic bark extract of *Terminalia Paniculata* Roxb. at a dose of 200, 400 and 600 mg/kg BW for 21 days. Authors showed that 400mg/kg and 600mg/kg alcoholic bark extract result in a significant reduce in the blood glucose levels in oral glucose tolerance test whereas 200mg/kg showed little effect. So, good effect of *T. arjuna* oral administration at a concentration of 500 mg/kg BW in comparison of 250mg/kg BW is logical.

Insulin tolerance test is an effective diagnostic marker for type-I and II diabetes. In type-II diabetes, the insulin resistance is the main characteristic, as the outer source of insulin will not rapidly normalize the oral load of glucose due to the non-functionality/ non-response of insulin and consequently, blood glucose will increase with time after oral administration of glucose. A similar pattern was observed in the present study, when glucose was administered orally with simultaneous injection of insulin, it was not restricted to post-prandial hyperglycemia in type-II diabetic control rats, which confirms the insulin-resistant characteristics of the type-II diabetic animals. Feeding acetone extract of *T. arjuna* bark exhibited a higher response to the outer source insulin and inhibited the postprandial hyperglycemic peak in these animals. The effects of feeding 500mg/Kg BW of *T. arjuna* bark acetone extract were more efficacious in comparison to the 250mg/Kg BW. Moreover, the feeding of glimepiride also showed higher efficacy in delaying the attainment of insulin-resistant stage in comparison to 250mg/Kg BW of *T. arjuna* bark extract treatments. These results clearly indicated that the feeding of 500mg/Kg BW of *T. arjuna* bark acetone extract significantly ($P<0.05$) attenuated the achievement of insulin resistance in type-II diabetic rats. Similarly, Lian *et.al.*, 2007[24] reported impaired glucose tolerance of diabetic control mice in comparison to the normal. Authors reported after the insulin injection, normal mice showed a sensitive response which was reflected in immediate decrease of blood glucose level but in contrast diabetic control mice showed a distinct delay decrease of the blood glucose level. Satoh *et.al.*, 2005[25] performed insulin tolerance test on 6 h fasted wistar rats and indicated glucose-lowering effect in Adv-Adipo (high viral dosage) rats compared with Adv-LacZ controls at 30, 45, and 60 min. In the present study diabetic rats showed the higher resistance towards exogenous insulin suggest low expression of insulin receptor impairment in insulin receptor. This could be one of the reasons of observed higher blood glucose level in diabetic control rats as compared to normal rats. Feeding arjuna bark acetone extract to diabetic rats increased pancreatic beta cell activity and blood insulin level. Increased plasma insulin level in diabetic rats could participate in increased glucose uptake by cells and decreased cytotoxicity and increased receptor expression. This could be one of the reasons of better insulin tolerance of diabetic rats fed with arjuna bark acetone extract.

5. Conclusion

From the present study it has been concluded that acetone extract of *T. arjuna* bark have higher antioxidative power than the methanolic extract and it exhibits anti-hyperglycemic activity on Type-2 diabetic albino rats. The blood glucose level 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 for 60 days which reduces the diabetic complications in albino rats . The acetone extract of *Terminalia arjuna* bark have tannins, alkaloids, triterpenoids, flavonoids which exhibit antioxidant power and hypoglycemic properties.

6. References

1. Barcelo A and Rajpathak S. Incidence and prevalence of diabetes mellitus in the Americas. American Journal of Public Health 2001; 10: 300-308.
2. Seth SD and Sharma B. Medicinal plants in india. Indian J. Med. Res. 2004; 120: 9-11.
3. Dey L, Anoja SA, Yuan CS. Alternative therapies for type 2 diabetes. Alternative Med. Rev. 2002; 7: 45–58.
4. Tripathi VK, Singh B, Tripathi RK, Upadhyay and Pandey VB. *Terminalia arjuna* its present status. Oriental J of Chem. 1996; 1: 1–16.
5. Handa SS and MK Kaul. Supplement to cultivation and utilization of medicinal plants. In: RRL. CSIR Publication, Jammu Tawi. 1996; 334 -348.
6. Moulisha Biswas, Kaushik Biswas, Tarun K. Karan, Sanjib Bhattacharya, Ashoke K. Ghosh, and Pallab K. Haldar. Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf. Journal of Phytology 2011; 3(1): 33-38.
7. Wang HJ, Jin YX, Shen W, Neng J, Wu T, Li YJ and Fu ZW. 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. 2007; 16 (1). 412-217.
8. AOAC. Official methods of analysis, 11th Edn. Association of Official Agric. Chemists, Washington, D.C. 1970: 250, 536, 858.
9. Sofowora, A. Screening Plants for Bioactive Agents. In: Medicinal plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria 1993: 134 – 156.
10. Benzie FF and Strain JJ. 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 1999; 299: 15-23.
11. Tran TT, Gupta N, Goh T, Naigamwalla D, Chia MC, Koohestani N, Mehrotra S, McKeown-Eyssen G, Giacca A and Bruce WR. Direct measure of insulin sensitivity with the hyper-insulinemic-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. 2003; 12(1): 47-56.
12. Shapiro K and Gong WC. Natural products used for diabetes. J. Am. Pharm. Assoc. 2002; 42(2): 217-226.
13. Banting FG and Best CH. The Internal Secretion of the Pancreas, Journal of Laboratory and Clinical Medicine. 1922; 7(5): 465-480.
14. Ribnicky DM, Poulev A, Watford M, Cefalu WT, Raskin I. Antihyperglycemic activity of Tarralin, an ethanolic extract of Artemisia dracunculus L. Phytomed. 2006; 13: 550–557.
15. Mythili P, Parameswari CS and J Dayana. Phytochemical analysis of the bark extract of *Terminalia arjuna* and its cardioprotective effect. 2nd National level students conference on nascent technologies in biomedical, Electrical engineering and communications (NT-BEECOM'12). 6th August, 2012; 40. Vol. 1: S 8.
16. Ragavan B and Krishnakumari S. Antidiabetic effect of T. arjuna bark extract in alloxan induced diabetic rats. Indian Journal of Clinical Biochemistry 2006; 21(2): 123-128.
17. Khanna AK, Chander. R and Kapoor NK. T. arjuna an ayurvedic cardiotonic regulates lipid metabolism in hyperlipeaemic rats. Phyto Res., 1996; 10: 663-665.
18. Dolly Singh, Deepti Singh, Soon Mo Choi, Sun Mi Zo, Saet Byul Ki, and Sung Soo. Therapeutical effect of extracts of Terminalia chebula in inhibiting human pathogens and free radicals. Han International Journal of Bioscience, Biochemistry and Bioinformatics 2012, 2(3): 164-167.
19. Amira K, Aminah, A and Zuhair, A. Evaluation of bitter melon (Momordica charantia) extract administration in the antioxidant and free radical scavenging activities of plasma and liver in male rat. International Food Research Journal 2013; 20(1): 319-323

20. Wilson RD, Islam MS. Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes.. Int J Med Sci. 2013; 10(4): 377–381.
21. Nagappa AN, Thakurdesai PA, Venkat Rao N and Jiwan Singh. Antidiabetic activity of Terminalia catappa Linn fruits. Journal of Ethnopharmacology 2003; 88(1): 45–50.
22. Murali YK, Ramesh Chandra and Murthy PS. Antihyperglycemic effect of water extract of dry fruits of Terminalia chebula in experimental diabetes mellitus. Indian Journal of Clinical Biochemistry 2004; 19(2): 202-204.
23. Govinda Rao M, Sarita Kotagiri, Vrushabendra Swamy BM, Archana Swamy P. 2012 Anti-diabetic activity of hydro-alcoholic bark extract of Terminalia paniculata Roxb. in streptozotocin induced diabetic rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012; 3(3): 790-807.
24. Ji-hong Lian, You-Qing Xiang, Lei Guo, Wei-Rong Hu, Wei Ji and Bang-Qiang Gong. The use of High-fat/carbohydrate diet-fed and streptozotocin-treated mice as a suitable animal model of Type 2 diabetes mellitus. Scand. J. Lab. Anim. Sci. 2007; 34(1): 21-29.
25. Hiroaki Satoh, Audrey Nguyen MT, Maria Trujillo, Takeshi Imamura, Isao Usui, Philipp E. Scherer, and Jerrold M. Olefsky. Adenovirus-mediated adiponectin expression augments skeletal muscle insulin sensitivity in male Wistar rats Diabetes 2005; 54: 1304-13013.