Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2014, 6 (3):102-109
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Antidiabetic activity of *Artocarpus heterophyllus* rag extract studied in high fat fed- low dose STZ induced experimental type 2 diabetic rats

E. R. Suchithra and S. Subramanian

Department of Biochemistry, University of Madras, Guindy Campus, Chennai, India

---

ABSTRACT

Jack fruit, the largest tree born fruit in the world, belongs to the family Moraceae and genus Artocarpus. Various parts of the plant have been reported to possess antibacterial, anti-inflammatory, antioxidant and immunomodulatory properties. The whitish yellow filament like structures present in the fruit is called "rags" which are actually unfertilized flowers that could not develop in to seeds. The rags are widely used in the Indian traditional medicine for the treatment of various ailments. In the absence of systemic reports in the literature, the present study was aimed to evaluate the antidiabetic potential of the Artocarpus heterophyllus rags in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats. Phytochemical screening of the rag extract was performed. Diabetic rats were treated with Artocarpus heterophyllus rag extract at a dosage of 300 mg/kg b.w daily for 30 days. Metformin (200 mg/kg. b.w) was used as a reference drug. The levels of fasting blood glucose, plasma insulin and HbA1c were also estimated. Intraperitoneal insulin tolerance test was performed. The level of glycogen content in liver tissue was estimated. The activities of serum aspartate transaminase, alanine transaminase and alkaline phosphatase were assayed. The rag extract supplementation attenuated the elevated levels of glucose, glycosylated hemoglobin, AST, ALT and ALP. The insulin level was improved with an improvement in hepatic glycogen content of insulin resistant diabetic rats. The altered activities of glycogen metabolizing enzymes were normalized upon extract treatment. The rag extract improves insulin sensitivity which is evident from intraperitoneal insulin tolerance test. The results show that the rags of Artocarpus heterophyllus is non toxic and possess significant antidiabetic properties which might be attributed to the presence of biologically active ingredients present in the rags.

Keywords: *Artocarpus heterophyllus* rag; Type 2 diabetes; high fat diet; Streptozotocin; antidiabetic

---

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic beta cells leading to chronic hyperglycemia. The global burden of T2DM continues to rise as the worldwide prevalence of adult diabetes is projected to increase from 246 to 380 million people by 2025. The rising rates of obesity in youth have concurrently led to an increase in the prevalent rates of type 2 diabetes mellitus. Reducing the incidence of T2DM by preventing pediatric obesity through the implementation of lifestyle changes in the community should be the primary objective of healthcare systems [1]. The treatment and management of type II diabetes is mainly focused on glycaemic control and the international guidelines recommend reducing glycosylated hemoglobin (HbA1c) to 6.5–7% [2].

Nature is an extraordinary source of antidiabetic medicines [3]. Herbal medicines have traditionally played a major role in the management of diabetes in Asian countries for centuries [4]. Despite recent advances in anti-diabetic strategies, no strategy is clinically successful. Treating diabetics without undesirable side effects still remains a formidable challenge in drug research and development. Plants provide an extraordinary source of natural medicines for different diseases. Moreover, secondary metabolites of plant origin serve as an invaluable chemical library for drug discovery and current medicinal chemistry in the pharmaceutical industry.

Jackfruit is a dicotyledonous compound fruit of the jack tree, *Artocarpus heterophyllus lamn*. which belongs to the family *Moraceae* and genus *Artocarpus*. It is an evergreen, latex producing erect tree that grows up to 80 feet in height. The jackfruit tree is believed indigenous to the rain forests of the Western Ghats of India and now grow abundantly in many parts of southeast Asia [5]. Jackfruit tree produces the largest tree borne fruits in the world and a mature tree can yield anywhere between 50 to 200 fruits per annum [6-10]. At times the fruits may be as heavy as 45kg in weight and up to 36 inches in length and 20 inches in diameter [11]**.** It is the national fruit of Bangladesh and is considered to be an extremely important tree by the natives [12]. In south India, the jackfruit is a popular next to the mango and banana.

The jackfruit is a multiple fruit i.e composed of the coherence of multiple flowers. The fruit skin is extremely rough and thick with spines. The tree is monoecious i.e male and female flowers are on the same tree. Female flowers were larger than the male and the pedicel is quit thick. The interior of the fruit consist of large bulbs or arils each enclose a light brown seed. There may be 100 to 500 seeds in a single fruit.

Aggregate fruit or syncarp is formed by the enlargement of the entire female head [13]. The heavy fruit is held together by central fibrous core. Fruits take 3 to 6 months to reach maturity. It is formed by the fusion of multiple flowers in an inflorescence. The fruit arils with their seeds are embedded in rags which are actually unfertilized flowers that did not developed in to seeds. Rags have been known to possess a number of medicinal properties. However, most people always throw away rags after enjoying the arils taste. *A. heterophylus* was reported to contain various chemical constituents such as morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, artocarpesin, oxydihydroartocarpesin [14]. Many uses for the rags have been reported in traditional medicine for the treatment of various ailments.

In the absence of systematic reports in the literature, the present study was aimed to explore the antidiabetic activity of *Atrocarpus heterophyllus* rag extract in HFD fed low dose streptozotocin induced type 2 diabetic rats.

MATERIALS AND METHODS

Plant Material

Mature *Atrocarpus heterophyllus* fruits were collected from a tree in Minjur, Chennai, Tamilnadu, identified and authenticated by a qualified taxonomist and a voucher specimen was deposited at CAS in Botany, University of Madras, Chennai.

Preparation of plant extract

Mature *Atrocarpus heterophyllus* rags were selectively collected from the jack fruit, washed and dried in an air oven at 50˚C. The dried rags were powdered in an electrical grinder, which was then stored in an airtight brown container at 5° C until further use. The powdered rags were delipidated with petroleum ether (60 - 80° C) for overnight. It was then filtered and soxhalation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40 – 50° C under reduced pressure. The 100gm of dried powder of *Atrocarpus heterophyllus* L yields 26.4g.

Preliminary phytochemical screening

The ethanolic extract of *Atrocarpus heterophyllus* rags were subjected to qualitative phytochemical screening for the presence of major phytochemicals [15, 16].

Experimental animals

Male albino rats of Wistar strain weighing (160-180g) were procured from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed in spacious polypropylene cages lined with husk. The experimental rats were maintained in a controlled environment (12:12 ± 1h light/dark cycle) and temperature (30°C ± 2). Animals were acclimatized to standard husbandry conditions for one week to eliminate the effect of

stress prior to initiation of the experiments. The rats were fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water *ad libitum*. The experiments were designed and conducted in strict accordance with the current ethical norms approved by Ministry of Social justices and Environment, Government of India and Institutional Animal Ethical Committee guidelines.

High fat diet fed streptozotocin induced diabetes

The experimental rats were segregated into two dietary regimens by feeding either normal or high fat diet (HFD) for the initial period of two weeks. After two weeks of dietary manipulation, the groups of rats fed with HFD was injected intraperitoneally with a low dose of STZ (35 mg/kg b.w) dissolved in 0.1M cold citrate buffer, pH 4.5). One week after STZ injection, the rats were analyzed for blood glucose level. The rats with fasting blood glucose (FBG) >250mg/dl that exhibited random hyperglycaemia and glycosuria were chosen for further studies. The rats were allowed to continue to feed on their respective diets until the end of the experiments.

Toxicity and dosage fixation studies

The acute toxicity of *Atrocarpus heterophyllus* rags was studied in control rats according to OECD guideline 423. Graded doses of *Atrocarpus heterophyllus* rags dissolved in water and administered orally and the animals were observed continuously for the first 2 hours followed by every hour up to 6 hours and daily thereafter for fourteen days for any signs of morbidity, mortality and behavioral toxicity. *Atrocarpus heterophyllus* rags were found to be non-toxic up to 2 g/kg b.w.

A graded dose of Atrocarpus heterophyllus rags (100, 200, 300, 400, 500 mg/kg b.w) was administered to HFD + STZ induced diabetic rats for various periods of treatment. From the data obtained, the optimum dosage was fixed as 300 mg/kg b.w for 30 days. The animals were divided into four groups, comprising a minimum of six animals in each group as follows:

Group 1 – Control rats.
Group 2 – HFD+STZ (i.p. 35mg/kg b.w.) induced rats.
Group 3 – *Atrocarpus heterophyllus* rag extract (300 mg/kg b.w.) treated diabetic rats
Group 4 – Diabetic rats treated with metformin (200 mg/ kg b.w/ day) in aqueous solution orally for 30 days.

At the end of the treatment period, the rats were fasted overnight, anesthetized and sacrificed by cervical decapitation. The blood was collected with and without anticoagulants for plasma and serum separation, respectively.

Intraperitoneal insulin tolerance test

At the end of the experimental period, fasting blood samples were withdrawn through retro-orbital bleeding from the control and experimental groups of rats. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after the intraperitoneal administration of a bolus of insulin (2 unit/kg bw). All the blood samples were collected with EDTA for the determination of glucose by using glucose oxidase peroxidase/diagnostic enzyme kit (Span Diagnostic Chemicals, Surat, India) and the analysis was performed according to the manufacturer's instructions.

Biochemical parameters

Fasting blood glucose level was estimated according to the method of Sasaki et al., 1972 [17]. Plasma insulin was assayed using the Ultra-sensitive ELISA kit for rat insulin (Linco Research, St Charles, MO, USA). Glycosylated hemoglobin (HbA1c) levels were estimated according to the method of Nayak and Pattabiraman [18]. Urine sugar was detected using commercially available urine strips (Diastix (2804B); Manufactured by Siemens Ltd, Gujarat, India). The activities of serum transaminases and alkaline phospatases were assayed [19, 20]. The liver and muscle tissues were dissected out and washed with ice-cold saline for determination of glycogen content [21].

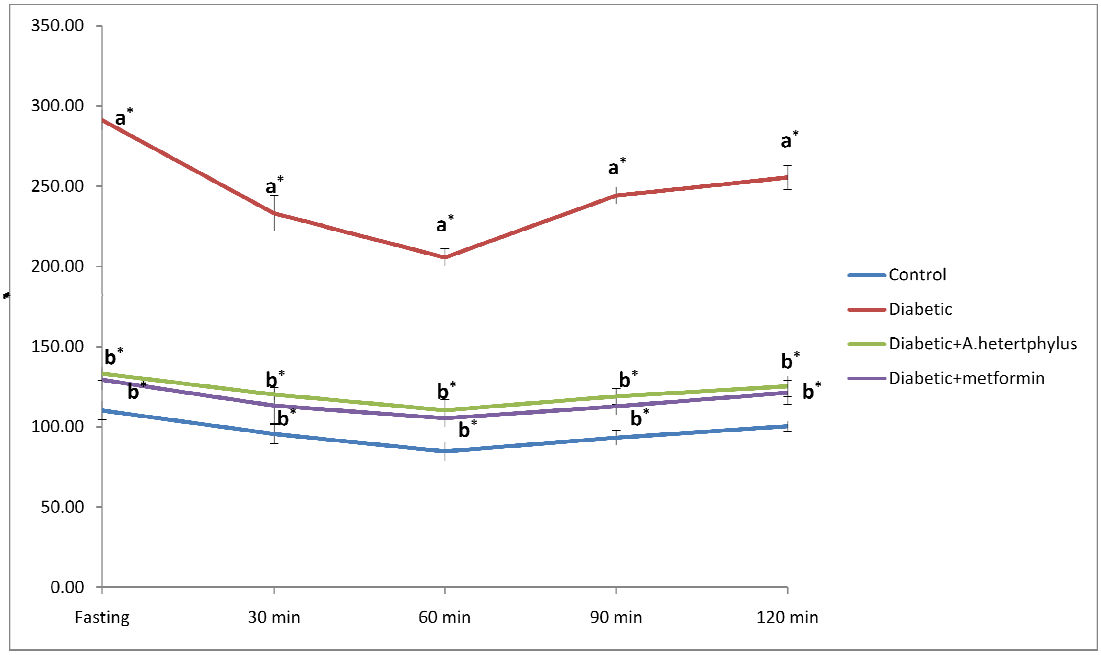
Statistical analysis

The results were expressed as mean ± S.E.M of six rats per group and statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS (version 16) program followed by LSD. Values were considered statistically significant when $p < 0.05$.

RESULTS

The effect of rag extract as well as metformin treatment on the level of blood glucose in the experimental groups of rats receiving an intraperitoneal insulin challenge is shown in Figure 1. The blood glucose level is significantly ($p<0.05$) reduced in diabetic rats treated with rag extract as well as metformin than that of the diabetic group of rats.

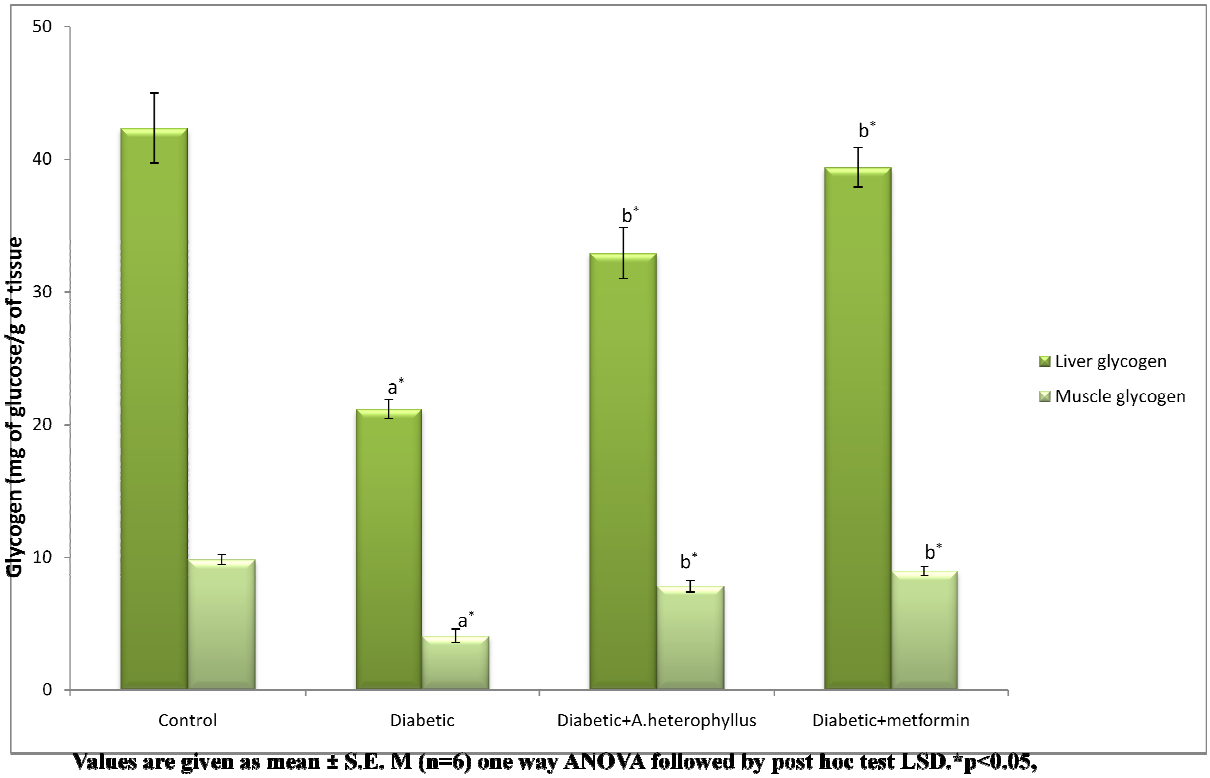
Figure 1: Effect of *A.heterophylus rag* extract on intraperitoneal insulin tolerance test in experimental groups of rats



Values are given as mean ± SEM for groups of six rats in each. One way ANOVA followed by *post hoc* test LSD. Values are statistically significant at * $p<0.05$; and are compared with [a] Control rats; [b] Diabetic control rats

Liver and muscle glycogen content of HFD-STZ diabetic control rats showed a highly significant decrease as compared to normal control (Figure 2). However, treatment with the rag extract and metformin improved the levels of glycogen content in liver and muscle tissues.

Figure 2: Effect of *A.heterophylus rag* extract on the levels of glycogen content in liver and muscle tissues in experimental groups of rats



Values are given as mean ± S.E. M (n=6) one way ANOVA followed by post hoc test LSD.*p<0.05, when compared with [a] Control rats; [b] Diabetic rats

Table 1 shows the phytochemical screening of the rag extract. The rag extract was found to contain biologically active ingredients such as flavonoids, alkaloids, glycosides, saponins and phenols.

Table 1: Phytochemical analysis of *A. heterophylus* rag extract

PHYTOCONSTITUENTS	INFERENCE
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Phytosterol	+
Triterpenoids	-
Glycosides	+
Anthraquinones	-
Phenols	+

The effect of rag extract on the levels of blood glucose, fasting plasma insulin and glycosylated hemoglobin (HbA1c%) in HFD-STZ diabetic rats is shown in Table 2. The levels of blood glucose and HbA1c% were found to be significantly elevated in diabetic rats as compared with normal control. Oral administration of rag extract to diabetic rats significantly improved the altered level. The levels of plasma insulin were moderately decreased in HFD-STZ induced diabetic rats. Diabetic rats treated with rag extract as well as metformin showed improved insulin level.

Table 2 The levels of blood glucose, glycosylated hemoglobin (HbA1c), plasma insulin and urine sugar in control and experimental groups of rats

Groups	Blood glucose	HbA1c	Insulin	Urine sugar
Control	88.41 ± 4.09	5.34 ± 0.33	15.80 ± 0.22	Nil
Diabetic	296.18 ± 7.43 [a*]	13.01 ± 0.44 [a*]	10.31 ± 0.28[a*]	+++
Diabetic + *A. heterophylus*	134.93 ± 6.73[b*]	7.30 ± 0.27[b*]	12.34 ± 0.45[b*]	Nil
Diabetic+ metformin	119.70 ± 6.51[b*]	7.00 ± 0.19[b*]	14.50 ± 0.45[b*]	Nil

Units: *mg/dl for blood glucose, % hemoglobin for HbA1c, µU/ml for plasma insulin, +++ indicates more than 2% sugar.*

Results are expressed as mean ±S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. Values are statistically significant at P<0.05. The results were compared with [a]Control rats, [b]Diabetic rats

Table 3 represents the effect of rag extract on the activities of serum AST, ALT and ALP in the experimental groups of rats. These pathophysiological indices in diabetic group of rats were significantly ($p < 0.05$) elevated as compared with control group of rats. Oral administration of rag extract to diabetic groups of rats significantly ($p < 0.05$) normalized the altered levels in comparison with control group of rats. The results are compared with the standard drug metformin.

Table3. Effect of *A. heterophylus rag* extract on the activities of serum transaminases and alkaline phosphatase in the control and experimental groups of rats

Groups	AST	ALT	ALP
Control	82.02 ± 2.66	21.08 ± 0.54	74.50 ± 1.07*
Diabetic	129.89 ± 2.35 [a*]	44.31 ± 1.15[a*]	144.35 ± 1.99[a*]
Diabetic + *A. heterophylus*	71.52 ± 1.30 [b*]	22.97 ± 1.63[*]	91.22± 1.73[b*]
Diabetic + metformin	84.79 ± 2.17 [b*]	18.86 ± 0.78[*]	71.01 ± 1.05[b*]

Enzyme activities are expressed as: AST and ALT - µmoles of pyruvate liberated/h/mg of protein, ALP - µmoles of phenol liberated/min/mg of protein.

Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD.

The results were compared with [a]Control rats, [b]Diabetic rats. Values are statistically significant at *P<0.05.

Table 4 represents the activities of glycogen synthase and glycogen phosphorylase in liver of control and experimental groups of rats. A significant (P<0.05) decline in the glycogen synthase activity and a concomitant increase in the activity of glycogen phosphorylase were observed in the liver tissues of diabetic group of rats. Oral treatment with the rag extract as well as metformin to HFD-STZ diabetic rats restored the activities of glycogen synthase, glycogen phosphorylase.

Table 4: The activities of glycogen synthase and glycogen phosphorylase in liver tissues of control and experimental groups of rats

Groups	Glycogen synthase	Glycogen phosphorylase
Control	828.21 ± 26.34	510.32 ± 32.15
Diabetic	439.27 ± 23.21 [a*]	792.77 ± 29.12 [a*]
Diabetic + *A. heterophylus*	688.10 ± 26.11 [b*]	653.80 ± 25.11[b*]
Diabetic + metformin	701.10 ± 31.32[b*]	629.24 ± 30.37[b*]

Units are expressed as: mg of glucose/g wet tissue for glycogen, µmoles of UDP formed/h/mg protein for glycogen synthase and µmoles Pi liberated/h/mg protein for glycogen phosphorylase.

Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats;

DISCUSSION

Metformin, an oral hypoglycemic agent belonging to biguanides is now widely used as one of the mainstays in the management of type 2 diabetes. Metformin reduces fasting plasma glucose concentration by reducing rate of hepatic

glucose production via gluconeogenesis and glycogenolysis. Metformin improves glycemic control as monotherapy and in combination with other oral antidiabetic agents, such as sulfonylureas and thiazolidine diones [22]. Hence in the present study metformin is used as a standard reference drug. The HFD rat model with low dose of STZ (35 mg kg^{-1}) clinically resembles the pathophysiological state of type 2 diabetes. Hence, HFD in combination with low dose of STZ (35 mg kg^{-1}) was chosen for generating the type 2 diabetic rat model.

Blood glucose is an index for the diagnosis of diabetes mellitus. In the present study, oral administration of rag extract decreased the levels of blood glucose and improved insulin sensitivity in HFD-STZ diabetic rats. The levels of plasma insulin were moderately decreased in HFD-STZ induced diabetic rats. Though the level was not decreased more, the insulin level in HFD-STZ diabetic rats could not stimulate glucose uptake in to the cells due to insulin resistant state. There was improved insulin sensitivity and a rise in insulin level in extract treated diabetic rats compared to control rats suggesting that rag extract exhibit significant insulin sensitization potential as well as improvement in the glucose homeostasis which may be probably due to improved pancreatic β-cell function. Urine sugar which was found in diabetic groups of rats was found to be absent in rats treated with rag extract indicating the amelioration of renal glycosuria which could be due to the improved glucose honeostasis.

Glycosylated hemoglobin is a biochemical marker that strongly correlates with the level of ambient glycemia during a 2- to 3-month period and is a more accurate and reliable measure than fasting blood glucose level [23]. The concentration of glycosylated hemoglobin strongly predicts the risk of eye, kidney and neural disease in diabetes mellitus and is regarded as a key target for the diagnosis and prognosis of diabetes-related complications [24]. Administration of rag extract significantly decreased the levels of glycosylated hemoglobin, suggesting an improved glycemic status.

The impaired activities of AST, ALT and ALP levels are associated with hepatic damage or the changes in the permeability of hepatocyte membrane due to oxidative stress. Elevated level of liver-function enzymes e.g., ALT and AST in serum are not only used for the identification of liver damage but also as a marker for the hepatic insulin resistance. A strong correlation exists between serum ALT level and insulin resistance but not for the AST, and it has been indicated as a predictor of T2D in human subjects [25]. Oral administration of the rag extracts to HFD-STZ induced diabetic rats decreased the activity of these hepatospecific enzymes indicating its non toxic nature.

Liver and skeletal muscle is the primary site of glucose disposal in the insulin-stimulated state. Glycogen is a storage form of carbohydrates. Excess glucose is converted into glycogen and stored as an energy fuel in tissues, predominantly in the liver and skeletal muscle. The glycogen level, the activity of glycogen synthase, glycogen phosporylase and responsiveness to insulin signaling are reduced in diabetes [26]. In the present study, the administration of the rag extract to diabetic rats improved the glycogen content and normalized the altered activities of glycogen metabolizing enzymes in both the liver tissues, which is due to improved glucose utilization and storage.

CONCLUSION

Treatment with *A. hetrophylus* rags improves glucose homeostasis and possesses insulin sensitizing effect in HFD fed-STZ-induced diabetic rats which is evident from the results of Insulin tolerance test, plasma insulin as well as blood glucose level. The results of the present study clearly indicate that oral treatment of the rag extract to diabetic rats increased the glycogen content as well as altered the activities of glycogen metabolizing enzymes suggesting the effective utilization of glucose which in turn may be due to improved insulin sensitivity. The rag extract treatment improve the glycemic status by modulating the key enzymes of carbohydrate metabolism in hepatic tissues of diabetic rats. The presence of various bioactive components such as flavonoids, alkaloids, saponins, glycosides and phenols may account for the observed pharmacological properties. Hence, A.hetrophyllus can be considered for use in the treatment and management of T2DM.

Acknowledgment
The author wish to acknowledge the University Grants Commission (UGC), New Delhi, India for the financial assistance provided in the form of UGC-BSR-RF.

REFERENCES

[1] V. Giampatzis, K. Tziomalos, *World J Diabetes*, **2012,** 12, 182-185.
[2] D.M. Nathan, J.B. Buse, M.B. Davidson, E. Ferrannini, *Diabetologia*, **2009**, 52, 17–30.
[3] C.L. Chang, Y. Lin, A.P. Bartolome, Y.C. Chen, S.C. Chiu, W.C. Yang, *Evid Based Complement Alternat Med*, **2013**, 378657.
[4] R. Uzayisenga, P.A. Ayeka, Y. Wang, *Phytother Res*, **2014.**
[5] A.K.M.M. Rahman, E. Huq, A.J. Mian, A. Chesson, *Food Chemistry*, **1995**, 52, 405−410.
[6] O.A. Alagiapillai, P.S. Kuttalam, V. Subramanian, M. Jayasekhar, *Madras Agricultural Journal*, **1996**, 83, 310−312.
[7] N. Haq, Jackfruit (Artocarpus heterophyllus). In J. T. Williams, R. W. Smith, & Z. Dunsiger (Eds.), Tropical fruit trees. Southampton, UK: Southampton Centre for Underutilised Crops, University of Southampton, **2006**.
[8] P. Narasimham, Breadfruit and jackfruit. In S. Nagy, P. E. Shaw, & W. F. Wardowski (Eds.), Fruits of tropical and subtropical origin: Composition. Properties and uses. (pp. 193−259) Florida: Florida Science Source Inc, **1996**.
[9] B.M.C. Reddy, P. Patil, S. Shashikumar, L.R. Govindaraju, *Karnataka Journal of Agricultural Sciences*, **2004**, 17, 279−282.
[10] H.M. Samaddar, Jackfruit. In T. K. Bose, & S. K. Mishra (Eds.), Fruits of India: Tropical and subtropical (pp. 638−649). Culcutta, India: Naya Prokash, **1985**.
[11] O. Prakash, R. Kumar, A. Mishra, R. Gupta, *Pharmaccognosy Review*, **2009**, 3, 353−358.
[12] T.K. Bose, Jackfruit. In B. K. Mitra (Ed.), Fruits of India: Tropical and subtropical naya prokas. Calcutta, India (pp. 488−497), **1985**.
[13] E.W.M. Verheij, R.E. Coronel, Plant resources of Southeast Asia No.2, Edible fruit and nuts, Wageningen, **1991**.
[14] A.V. Rama Rao, Mala Varadan, Venkataraman, *Indian J. Chem*, **1973,** 11, 298-299.
[15] J.B. Harborne, *Phytochemical methods*, Chapman and Hall Int., New York, Third Edition, **1998**.
[16] C.K. Kokate, *Pharmacognosy*, Nirali Prakasham, Mumbai, India, Sixteenth Edition, **2001**.
[17] T. Sasaki, S. Matsy, A. Sonae, *Rinsh kagaku*, **1972**, 1, 346-353.
[18] S.S. Nayak, T.N. Pattabiraman, *Clin Chem Acta*, **1981**, 109, 267-274.
[19] King J: The hydrolases-acid and alkaline phosphatases, *In: Practical clinical enzymology*, (Ed.) Van D. Nostrand Co, London, **1965**, pp.199-208.
[20] J. King, The transaminases: alanine and aspartate transaminases, *In: Practical Clinical Enzymology* (ed.) D.Van, Nostrand Co., London, **1965**, pp. 363–395
[21] M.A. Morales, A.J. Jabbagy, H.R. Terenizi, *Neurospora News*, **1973**, 20, 24–25.
[22] M.S. Frendell, N.B. Glazer, Y.E. Zhan, *Journal of Diabetes and its Complications*, **2003,** 17, 211-217.
[23] D.E. Goldstein, R.R. Little, R.A. Lorenz, J.I. Malone, D.M. Nathan, C.M. Peterson, *Diabetes Care*, **2004**, 27:S91-S93.
[24] J. Howlett, M. Ashwell, *Am J Clin Nutr*, **2008**, 87, 212S-216S.
[25] N.H. Cho, H.C. Jang, S.H Choi, H.R.Kim, H.K. Lee, J.C. Chan, S. Lim, *Diabetes Care*, **2007**, 30, 2566–2568.
[26] A.W. Thorburn, B. Gumbiner, F. Bulacan, P. Wallace, R.R Henry, *J Clin Invest*, **1990,** 85, 522–9.