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Original Article

Antioxidant, Anti-Inflammatory and Anti-diabetic Efficiency of Indian Medicinal Plants against Streptozotocin Induced Diabetes in Male Wistar Rats

Satish Kumar Vemuri, Rajkiran Reddy Banala, Kumar Katragunta, Vijaya Madhuri, Kiruthika Raju, Venkata Gurava Reddy Annapareddy, Pedda Venkata Subbaiah Goli*

ABSTRACT

Objective: The study was aimed to assess the anti-oxidative and anti-diabetic efficacy of few dietary supplements such as *Artocarpus heterophyllus* (Raw Jackfruit), *Zea mays* (Corn Silk), *Syzygium cumini* (Black plum), and Shilajit (Black asphaltum) in *in vitro* and *in vivo* models. The phytoextracts were compared with the known commercial diabetic drugs (Aminoguanidine, Glibenclamide and Insulin) in controlling Streptozotocin induced hyperglycemia, advanced glycation end-products (AGEs), hyperlipidemia and activation of inflammatory mediators (iNOS) in *in vivo*. **Methods:** Collection and preparation of phytoextracts, superoxide anion scavenging assay, phosphomolybdate assay (total anti-oxidant capacity), Hydroxyl radical scavenging assay, quantification of hemoglobin glycation, body weights, lipid profile, Pro-inflammatory gene expression, agarose gel electrophoresis and histopathology. **Results:** We found that natural extracts of *A. heterophyllus, S. cumini, Z. mays,* and Shilajit exhibited considerable antioxidant capacity and inhibited hemoglobin glycosylation in a dose-dependent manner in *in vitro*. In STZ-treated diabetic rats, the extracts showed clear effects on blood glucose and lipid levels, body weight and pro-inflammatory gene expression (iNOS). Histopathology of treated rat pancreas further supported our *in vivo* findings. Overall, our study indicates that the natural extracts we tested could be a useful source of antioxidant agents, functional foods and nutraceuticals, particularly in diabetes and related complications. **Conclusion:** The findings from our study showed reversal of the effects induced by Streptozotocin administration on phytoextracts supplementation in experimental diabetic rats and the therapeutic capacity was similar to that of commercial diabetic drugs, hence these extracts could serve as an alternate in management of diabetes and allied complications.

Key words: Medicinal plants, Streptozotocin (STZ), Glycation, Glycosylated hemoglobin, iNOS.

Key message: The present study relates to the effects of A. *heterophyllus, S. cumini, Z. mays*, and Shilajit extracts on glycation of hemoglobin (*in vitro* studies). The parameters such as body weights, biochemical (hyperglycemia and hyperlipidemia), molecular expression of pro-inflammatory gene (inducible nitric oxide (iNOS)) and morphological changes in the pancreas were studied in Streptozotocin induced diabetes in male Wistar rats. The extracts reversed the above mentioned parameters in experimental diabetic rats and correlate with the therapeutic capacity of the standard drugs (Glibenclamide, Insulin, Metformin and aminoguanidine).

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INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder and around 140 million people are suffering worldwide causing multiple organ dysfunction, mortality and morbidity.[1] Diabetes mellitus is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action process known as protein glycation.[1-2] suggested that glycation of protein (collagen and albumin) plays primary role in the development of chronic diabetic complications. Glycated proteins or products tend to form advanced glycation end-products (AGEs) causing irreversible structural and functional damage to the affected protein molecules.[2]

The physiological and biochemical catabolic processes in cells tend to generate the free radicals and other reactive oxygen species leading to oxidative stress.[3] Oxidative stress is defined as an imbalance between the generation of oxidants and anti-oxidant defense capacity of the body,[4] and is suggested as an underlying cause for cancer, diabetes, atherosclerosis and associated complications.[5] in humans which could be restricted by including natural anti-oxidants in regular diet or in daily regime.[6]

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Experimental animal models for Type 1 diabetes mellitus were developed by inducing Streptozotocin (STZ).[7,8,9] Alloxan.[10,11] etc. The administration of STZ can initiate ROS generation and leading to the loss of β-cells of islets of langerhans by necrosis and degranulation leading lower secretion of insulin in blood causing type 1 diabetes mellitus.[8] The β-cells destruction in Type1 diabetes is implicated on IL-1b and inducible nitric oxide synthase (iNOS).[12]

Currently, Glibenclamide, Insulin and Metformin (anti-hyperglycemic) and aminoguanidine (anti-glycation) are used in diabetes management.[13] The use of anti-diabetic drugs are limited due to loss of efficacy and side effects (i.e. hepatotoxicity, nephrotoxicity and depletion of anti-oxidants) on long term administration.[14] Hence, the pursuit for alternative and safe medication is ongoing. Natural extracts from several medicinal plant species were considered as an alternative sources for anti-oxidant and anti-diabetic molecules.[15] Many studies had reported the beneficial effects of plant extracts and their products in the management of diabetes.[16]

In the present study, we have made an attempt to validate the anti-hyperglycemic efficiency of the natural extracts of *Artocarpus heterophyllus* (Raw Jackfruit),[17] Corn Silk (*Zea mays*),[18] *S. cumin* (Black plum).[19,20] and Shilajit (Black asphaltum).[21] *in vitro and in vivo* models. *A.heterophyllus* contains high fibre content (~11 per cent), and also possess anti-diabetic and anti-oxidative properties.[17] *Zea mays* silk or Corn silk (*Z.mays silk*) extracts are known to exert anti-hyperglycemic properties. The *S. cumini* (black 1plum) bark, leaf and seed extracts were used in Ayurveda and Unani systems of medicine for treating diabetes, inflammation, dental issues, digestive disorders and dermatitis.[19] Shilajit is a complex mixture of organic humic substances (Lava rocks) found in Himalayas and Hindukush ranges of the Indian subcontinent which possess medicinal properties.[21] Processed Shilajit provides protection against STZ induced oxidative damage and diabetes in rats.

We investigated the anti-oxidant and anti-glycation efficiency of natural extract in *in vitro and in vivo.* Type 1 diabetic rat model was developed by Streptozotocin (STZ) administration and its toxic effects were ameliorated with the administration of natural extracts. Our biochemical, molecular and histopathological results provide a rational basis to consider the therapeutic and supplementary role of the natural product extracts in management of diabetes and its allied complications.

MATERIALS AND METHODS

Collection and Preparation of Extracts (Table 1)

Fruits of *A. heterophyllus*, *S. cumini* and Corn silk (*Z.mays silk*) were obtained from local vegetable markets. The collected fruits and *Z.mays silk* were cut into small pieces and blended by juicer and then the blended material was mixed with 50% of ethanol and 50% of double distilled water. The mixture was kept on the rotary shaker for 48 hrs. Extract was filtered using Whatmann No.1 filter paper and centrifuged at 5000g for 15 min and the supernatant was collected and concentrated using rotary evaporator. Collected test material was lyophilized. The lyophilized powder was stored at 0-4°C for further use. Purified extracts of Shilajit known to have anti-diabetic effects was provided to us by an ayurvedic *practitioner (Dr Prasad, Ayurveda clinic, Hyderabad)*

Superoxide anion scavenging (SAS) assay

The superoxide anion scavenging (SAS) efficiency of the natural extracts was done using the modified protocol of Ravishankara *et al.*[22] Ascorbic acid was used as standard and the absorbance was measured at 560 nm. The scavenging ability of the plant extract was determined by the following equation:

$$Scavenging\ activity\ (\%) = \frac{(1\text{- absorbance of sample})}{(\text{absorbance of the control})} \times 100$$

Phosphomolybdate assay (total anti-oxidant capacity)

The total anti-oxidant capacity of the natural extracts was determined by Phosphomolybdate method using ascorbic acid as a standard.[23] Ascorbic acid was used as standard and the absorbance was measured at 765 nm. The anti-oxidant capacity was estimated using following formula:

$$Antioxidant\ effect\ (\%) = \frac{(\text{control absorbance} - \text{sample absorbance})}{(\text{control absorbance})} \times 100$$

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging capacity was determined using modified method of Kwon *et al.* (2010).[24] The ascorbic acid was used as standard and absorbance was measured at 532 nm. The scavenging activity on hydroxyl radical was calculated as follows:

$$Scavenging\ activity\ (\%) = \frac{(1\text{- absorbance of sample})}{(\text{absorbance of the control})} \times 100$$

In vitro studies

Evaluation of Hemoglobin Glycation

Hemoglobin glycation was estimated by the method of *Adisa et al. (2004)*.[25] The concentrations of glycated hemoglobin were estimated using colorimeter at 443nm after an incubation period of 24 and 48 h.

In vivo studies

Experimental animals

Wistar male rats aged 6 months, weighing between 180-200 g and the standard pellet feed were procured from Jiva Lifesciences, Uppal, Telan-

Table 1: Comparison of free radical scavenging efficiency of various plant extracts.

Scientific name	Common name	Extracted part
Plants		
Artocarpus heterophyllus	*Jack Fruit*	*Tender raw jack fruit*
Syzygium cumini	*Black plum or Jamu or jambolan*	*whole ripe fruits*
Zea mays	*Maize or corn*	*Corn silk*
Other extracts		
Black asphaltum	*Shilajit/Silajatu/ Momio/rock conqueror*	

gana, India. The animals were maintained as per the guidelines CP*Z. mays silk* EA (**CP*Z.mays silk* EA/IAEC/JLS/003/03/15/002**), India.

Experimental induction of diabetes

Type1 diabetes was induced through single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (70 mg/ kg b.w.) in 0.1 M citrate buffer (pH = 4.5) to overnight starved rats.[26] Diabetic rats were permitted to drink 20% glucose solution overnight to overcome the initial drug induced hypoglycemic death. The blood glucose level was measured after three days, and rats with glucose levels >250 mg/dL were considered as diabetic. At the time of induction, control rats were injected with 0.2 mL of vehicle (0.1 M citrate buffer, pH 4.5) alone.

Experimental design

42 males were divided into 7 groups (6 normal and 36 STZ diabetic existing rats) were used. They were separated into seven groups of 6 rats each. Group I: Control rats (were given 0.5 mL of 0.9% saline orally for 35 days). Group II: Diabetic group (STZ 70 mg/kg b.w.). Group III: Diabetic rats were given Glibenclamide (600 µg /kg b.w. dissolved in 0.5 mL of 0.9% saline) for 35 days. Group IV: Diabetic rats were given *A. heterophyllus* (200 mg/kg b.w. dissolved in 0.5 mL of 0.9% saline) orally for 35 days. Group V: Diabetic rats were given *S. cumini* (200 mg/kg b.w. dissolved in 0.5 mL of CMC) for 35 days. Group VI: Diabetic rats were given Corn silk (*Z.mays silk*) (200 mg/kg b.w. dissolved in 0.5 mL of 0.9% saline) for 35 days. Group VII: Diabetic rats were given Shilajit (200 mg/kg b.w. dissolved in 0.5 mL of 0.9% saline) for 35 days. Towards the end of the study (35 days), the animals were euthanized by ketamine (24 mg/kg/body). To investigate the effects of anti-diabetic drugs, blood was collected from the diabetic rats every alternate day for 5 weeks and they were uniformly grouped for glycosylated hemoglobin and lipid levels among groups and for each model.

Determination of Body Weight

During the study period of 35 days, body weights of experimental groups were determined on the 1st day and 35th day after induction of diabetes by administering STZ.

Estimation of biochemical parameters

Glucose level in plasma was determined by glucose oxidase-peroxidase-hexokinase method using auto-analyzer (AU480-Beckman coulter. inc) at 540 nm against reagent blank.[27,28] The lipid profile was determined on regular intervals i.e. day 1, 7, 14, 21 and 35 before the animals were euthanized. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) in serum was determined by using commercial kits on automatic bioanalyzer (AU480-Beckman coulter. inc).[26]

iNOS gene expression analyses using real- time PCR

The mRNA expression levels of iNOS carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR).[12] Briefly Total cellular RNA was isolated from tissue using RNA sure mini kit according to manufacturer's protocol. cDNA was synthesized from 2 µg of total isolated RNA by incubation for 1h at 42°C with M-MLV reverse transcriptase (Thermo Scientific) and oligo (dT) 18 primer according to the manufacturer's instruction. Then 2.5µl of the reaction mixture was subjected to PCR for amplification of iNOS and GAPDH using specifically designed primers for these genes and genes were co-amplified in each reaction. The primers were designed for iNOS (Forward: TCACGACACCCTTCACCACAA, Reverse: CCATCCTCCTGCCCACTTCCTC), and GAPDH (Forward: TGAGGTGACCGCATCTTCTTG, Reverse: TGGTAACCAGGCGTCCGATA), were analyzed respectively. The PCR reactions was carried out in a final volume of 10 µl containing 1x PCR buffer and Emerald AMP GT PCR Master mix 2x premix (Takara clonetech) and 0.4 µM of each primer. The template was denatured for 5min at 94°C, followed by amplification cycles at 94°C for 1min, 66.5°C (for iNOS, 61°C and 59°C for GAPDH) for 1min and 72°C for 1.20 min, and terminated with an additional extension step for 8 min at 72°C. The PCR products were visualized using 1.5% agarose gel electrophoresis with ethidium bromide staining. In negative control, template cDNA was replaced by DEPC water.

Histopathology studies

The pancreatic tissues of the experimental rats were fixed in 10% formaldehyde; tissues were dehydrated by using different concentrations of ethanol and embedded in paraffin. Pancreatic tissue sections (5 µm thick) were acquired utilizing rotary microtome, and afterward rehydrated. Sections were then stained by hematoxylin-eosin (H and E) and viewed under the light microscope and shot by photomicrography.[7]

Statistical analysis

Statistical analysis of the data was performed using two-way ANOVA and followed by student T-test. Difference between the values was considered significant *p<0.5, and highly significant**p< 0.05.

RESULTS

Anti-oxidative properties

Figure 1 represents the Color code: 1 Red: Superoxide free radicals (OD at 560 nm), 2 Blue: Total antioxidant (OD at 765 nm), 3 yellow: Hydroxyl free radicals (OD at 532 nm), for different estimations, n=5, *p values <0.05.

Free radical scavenging activities of natural extracts at different concentrations EC50 values (µg/ml) of radical scavenging. On comparing the free radical scavenging and total antioxidant activity of all the extracts. It was observed that the total anti-oxidant capacity of *A. heterophyllus* was high in comparison to *S.cumini, Z.mays silk* and Shilajit. *S. cumini* showed higher SAS activity in comparison to other. The hydroxyl radicals scavenging capacity of Shilajit was marginally more than *S.cumini* and *Z.mays silk*, but significantly higher than *A. heterophyllus*. (Figure 1)

A. heterophyllus showed the maximum high anti-oxidant capacity of 101%, superoxide radical scavenging capacity of 85% and the hydroxyl radical scavenging activity of 22%. *S. cumini* showed an anti-oxidant capacity of 92%, superoxide free radical scavenging capacity of 90% and hydroxyl radical scavenging activity of 43%. *Z.mays silk* extract showed anti-oxidant capacity of 96%, superoxide radical scavenging capacity of 85% and hydroxyl radical scavenging activity of 42%. Shilajit extract showed total anti-oxidant capacity of 97%, superoxide radical scavenging activity of 60% and hydroxyl radical scavenging activity of 43%. All the effects were tested at the lowest dose of the extracts (Table 2).

Anti-glycation properties

The anti-glycation property and efficiency of natural extracts is represented in Figure 2. Our results demonstrate that the natural extracts had successfully restricted the glycation of hemoglobin. The exposure of hemoglobin to 2% glucose over a period of 24h and 48h increased the glycosylation of hemoglobin. The hemoglobin samples with 2% glucose when incubated with different concentration of the commercial drugs and natural extracts showed glycation inhibitory activity. The extracts of *S. cumini* and Shilajit showed better anti-glycation effects when compared to Insulin, glibenclamide, *A. heterophyllus* and *Z.mays silk* (n=5, **P < 0.05) (Figure 2).

Among all the extracts used in the study, extracts of *S. cumini* and Shilajit were the most effective in decreasing glycation of hemoglobin. The anti-glycation effects of natural extracts were comparable to that of anti-

Table 2: Free radical scavenging efficiency of various plant extracts (n=5, p values <0.05*).

Plant extracts/ chemical	Superoxide radical scavenging assay	Phosphomolybdate assay (Total antioxidant assay)	Hydroxyl radical scavenging assay
***Artocarpus heterophyllus*/** Ethanol	85±2%*	101±1%*	22±3%
***Syzygium cumini*/** Ethanol	90±1%*	92±2%	43±3%
***Zea mays*/**Ethanol	85±3%*	96±1%*	42±2%
Black asphaltum/ Shilajit/ Ethanol	60±2%	97±3%*	43±1%*

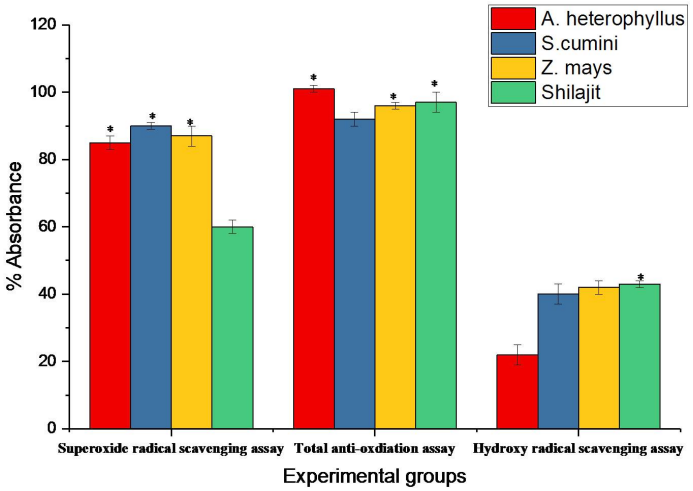

Figure 1: Comparison of free radical scavenging efficiency of various plant extracts.

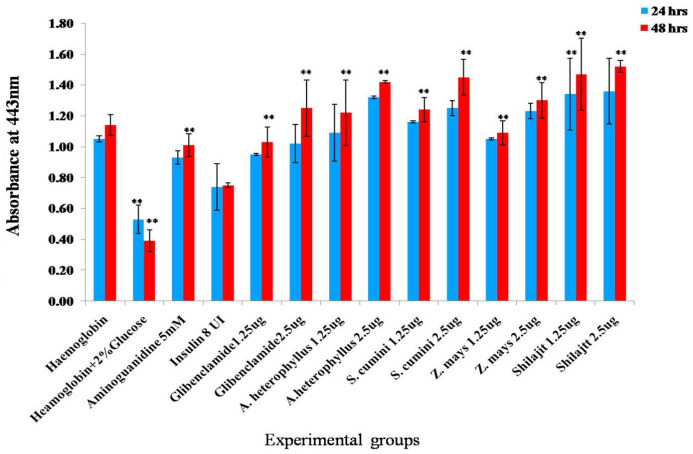

Figure 2: Glycation of hemoglobin: reduction in glycation of hemoglobin, when 2% of glucose was added to the blood samples in presence and absence of natural fruit extracts. (P < 0.05).**

diabetic drugs like human insulin and Glibenclamide; we observed that *S. cumini* and Shilajit extracts of 50 µg/mL showed higher activity than reference drugs i.e. Aminoguanidine, Insulin and Glibenclamide (Table 3).

Measurement of Body weights (BW)

After 35 days of study period, a significant decrease (n=5, **P < 0.05) was observed in the body weight (167.55 ± 3.96 g) of the STZ induced diabetic rats when compared with control group (234.44 ± 4.42 g). However, on glibenclamide (600 µg/kg.bw) administration to diabetic rats prevented this significant loss of body weight and helped in recovery of the body weights to near normal levels i.e. 226.00 ± 4.50 g (n=5, **P < 0.05). The administration of different natural extracts (*A.heterophyllus, S.cumini*, Shilajit and *Z.mays silk*) to experimental diabetic rats have shown similar efficiency in recovery of the body weights (220.11 ± 2.41; 221.25± 5.25; 218.55± 2.25; and 189.35 ± 4.25g), (n=5, **P < 0.05), but comparatively *Z. mays* silk extract showed less weight gain. (Table 4).

Estimation of Blood glucose

The effect of natural extracts and insulin on blood glucose levels in experimental diabetic rats were presented in Figure 3. In diabetic control rats, a significant rise (n=5, **P < 0.05) in blood glucose levels was observed i.e. more than 50%. The experimental diabetic rats, on administration with natural extract (200 mg/kg bw) significantly (n=5, **P < 0.05) reduced blood glucose levels by more than 50%, which was comparable to the effect of Glibenclamide in reducing the blood glucose levels (n=5, **P < 0.05). However, a significant reduction in blood glucose levels was observed in all the experimental rats on treatment with natural extracts, but it was observed that *Z.mays silk* extracts efficiency in controlling the blood glucose was very less when compared to the other extracts (Shilajit > *A. heterophyllus* > *S.cumini*) and Glibenclamide. Findings from the above parameter, indicates anti-glycemic properties of all the natural extracts (Figure 3).

Hypolipidemic property of Natural extracts

The effect of natural extracts and Glibenclamide on lipid profile in experimental diabetic rats were presented in Figure 4. In diabetic control rats, a significant rise (n=5, **P < 0.05) in lipid levels was observed with increased time duration i.e. more than 50%. In experimental diabetic rats on administration with natural extracts (200 mg/kg bw) significantly (n=5, **P < 0.05) reduced the levels of TGL, LDL, VLDL and increased levels of HDL, which was comparable to the effect of Glibenclamide in reducing the lipid levels (n=5, **P < 0.05). However, a significant reduction in lipid levels were observed in all the experimental rats treated with natural extracts, but on comparison, we found that *S.cumini* > Shilajit > *A. heterophyllus* > *Z.mays silk*. Findings from the above parameter, it is

Table 3: Glycation inhibition capacity of plants extract with different concentrations (represented as approximate percentages) at higher concentrations, extracts showed significant decrease in glycation of hemoglobin.

Drugs	200 µl (10µg/mL)	100 µl (5 µg / mL)	50 µl (2.5 µg / mL)	25 µl (1.25 µg /mL)
Aminoguanidine (5mM/100uL)	------	<50%	------	------
Glibenclamide 50 µg/mL	>50%	------	------	------
Insulin 8Ul	>50%	<50%	------	------
Natural Extracts				
Artocarpus heterophyllus (50ug/mL)	>50%	<50%	------	------
Zea mays (50ug/mL)	------	------	<50%	<50%
Syzygium cumini (50ug/mL)	------	------	>50%	>50%
Black asphaltum/Shilajit (50ug/mL)	------	------	>50%	>50%

Table 4: Effect of natural extracts and glibenclamide on body weight in experimental groups.

	Mean body weight (gm)		
Groups	Initial weight	1st week (After administration of STZ)	5th week
Control	228.43 ± 5.07	229.43± 5.07	234.44 ± 4.42
Streptozotocin (STZ)	229.23 ± 6.21	176.23 ± 6.21	167.55 ±3.96**
Glibenclamide	228.14 ± 6.33	186.14 ± 5.33	226.00 ± 4.50**
Artocarpus heterophyllus	227.65 ± 4.42	176.65 ± 5.42	220.11 ± 2.41**
Syzygium cumini	232.15±4.25	195.45±5.25	221.25±5.25**
Zea mays	223.25±7.25	189.29±5.25	179.35±4.25
Black asphaltum/Shilajit	222.45±3.25	201.15±5.25	218.55±2.25**

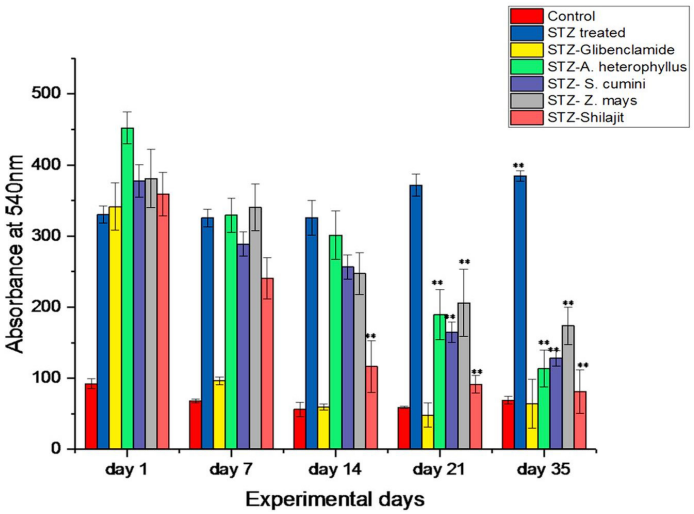

Figure 3: Effect of natural extracts and glibenclamide on blood glucose levels in STZ induced diabetes model in rats (Data was analyzed by two-way ANOVA followed by Student T-test (n = 5); **P < 0.05 as compared to control animals and diabetes group at respective time points).

evident that the natural extracts do have anti-lipidemic properties and they are comparable to that of reference drugs (Figure 4).

Histopathology findings

The histopathological findings: A) Control showed normal acinar cells in non-glandular pancreas (Black arrow) and there no signs of degeneration or necrosis noticed (red arrow). B) The STZ induced tissues showed mild fatty degeneration in the acinar cells of pancreas and fibrosis and hyperplasia was observed in ductular epithelial cells (red arrow) and periductular pancreas (black arrow). C) The tissue of Glibenclamide administered showed normal beta cells and acinar cells and mild hyperplasia was noticed in ductular epithelial cells (green arrow). D) The *A. heterophyllus* administration has reversed the degenerative effects induced by STZ, the acinar cells appeared normal and there were no signs of degeneration or necrosis (red arrow). The beta cells in islets of pancreas showed mild atrophy (black arrow) and periductular fibrosis (green arrow). E) Atrophy of beta cells in the islets of pancreas (black arrow) and mild degeneration noticed in the ductular region of pancreas (red arrow) was observed in *S. cumini* treated samples. F) The *Z. mays* silk treated was not able to counteract the STZ induced mild fatty degeneration in the acinar cells of pancreas and fibrosis and hyperplasia in ductular epithelial cells (red arrow) and periductular pancreas (black arrow). G) Shilajit treated samples showed normal acinar cells in non-glandular pancreas (Black arrow) and there were no signs of degeneration or necrosis noticed (red arrow) (Figure 5).

Molecular analysis of pro-inflammatory gene

Figure 6 shows the expression status of pro-oxidative and pro-inflammatory genes (iNOS) in pancreatic tissues of diabetic rats which received simultaneous treatment of natural extracts along with STZ. The molecular weights of iNOS and GAPDH are 250 bps and 104 bps respectively.

Expression of the iNOS was compared to that of GAPDH gene (reference control). STZ-treated rats were fed simultaneously with natural extracts (200 mg/kg.bw) clearly showed anti-oxidative and anti-inflammatory effects and which was verified by iNOS gene expression in the pancreatic tissue. We observed that extracts of *A. heterophyllus*, *S. cumini* and Shilajit down regulated iNOS expression, whereas *Z. mays* silk extract did not show an effect on iNOS expression (Figure 6).

DISCUSSION

Literature studies, suggests decline in the levels of natural antioxidants is a major concern in incidence of chronic diseases such as diabetes, cancers and cardiomyopathies.[16] Over decades researchers had focused on maintaining the levels of antioxidants for combating the free radical induced stress. The anti-oxidant capacity exhibited by plant prod-

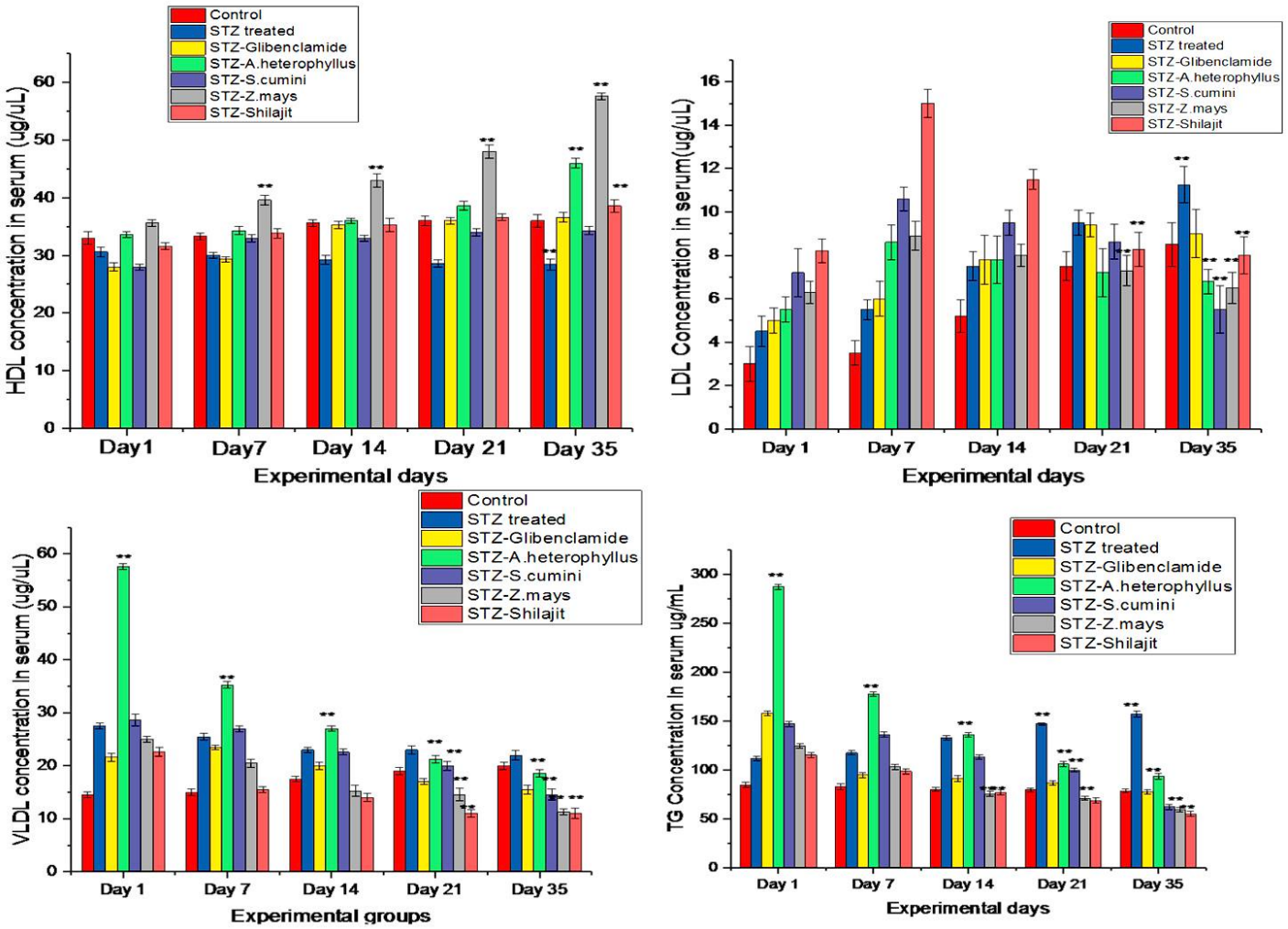

Figure 4: **Hypolipidemic effect of natural extracts against STZ induced alterations.**

ucts is mainly due to redox properties of natural phenolic compounds such as flavonoids, polyphenols, tannins, terpenes, and their free radical scavenging activity in neutralizing the singlet or triplet oxygen, and peroxides.[6] Antioxidant supplementation has significantly decreased the elevated hyperglycemia and hyperlipidmia in Streptozotocin induced diabetic rats.[26] Several studies reported about the anti-diabetic and anti-lipidemic effects of proanthocyanidin, flavonoid and dietary fiber contents present in natural extracts.[16]

The present study shows an *in vitro* and *in vivo* evaluation of the anti-glycemic, anti-lipidemic, anti-glycation and anti-oxidant properties present in natural natural extracts. The *A. heterophyllus* extract exerted significant antioxidant property in comparison to the other extracts. S.cumini extract exerted better superoxide radical scavenging property than other extracts. Hydroxyl radical scavenging activity of Shilajit was relatively high when compared to the other extracts. (Table 1 and Figure 1).[16] Our *in vitro* results clearly indicate the anti-glycation efficiency of natural extracts of *A. heterophyllus, S. cumini, Z.mays silk,* and Shilajit in a dose-dependent fashion. Shilajit exhibited high anti-glycation activity comparable to the reference drugs (Glibenclamide, Insulin and Aminoguanidine) *in vitro* (Table 2 and Figure 2).[3]

Findings from our *in vivo* study, demonstrate a significant reduction in glycemic index and significant weight gain in experimental rats after the oral administration of various natural extracts (200 mg/kg) and

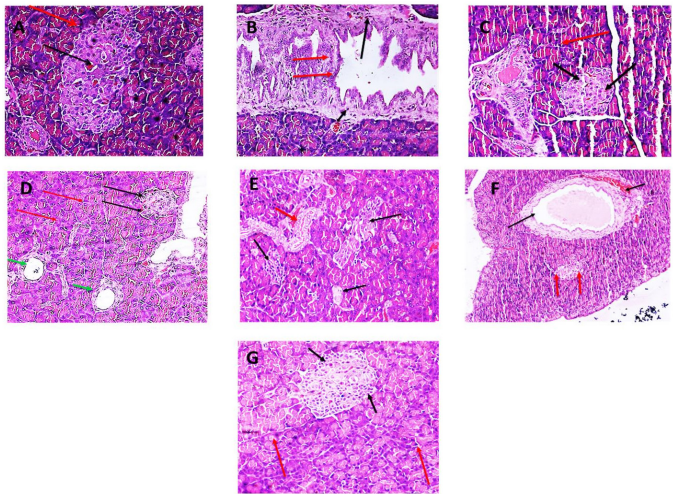

Figure 5: **Amelioration of STZ-induced histopathological alterations in pancreatic tissue exposure to natural extracts.**

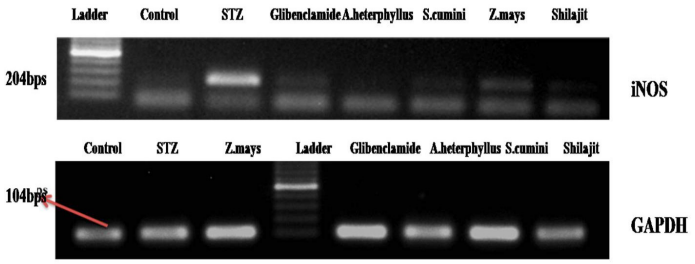

Figure 6: Anti-oxidative and anti-inflammatory property of natural extract against STZ induced inflammation.

Glibenclamide (600 µg /kg b.w) over a period of 5 weeks. (Table 4 and Figure 3).[18,27]

The diabetic control rats showed hyperlipidemic condition when compared to the control and other experimental groups. Administration of Glibenclamide (reference drug), *A. heterophyllus, Z.mays silk*, *S. cumini* and Shilajit extracts to the experimental diabetic groups showed ameliorative effects and the lipid profile was similar to that of control group. High Density Lipoproteins (HDL) levels were found to be significantly higher in the groups receiving natural extract and glibenclamide than diabetic control group and the levels were on par with negative control group. (Figure 4).[26]

The histopathological findings from H and E stained pancreatic tissue of STZ treated diabetic rats with and without administration of natural extracts and Glibenclamide. Protective effects of natural extracts and glibenclamide was observed in the experimental groups. STZ treated group showed mild fatty degeneration in the acinar cells, fibrosis and hyperplasia in ductal epithelial and periductal cells of pancreas. *A. heterophyllus and Shilajit* treated groups showed normal acinar cells reversed the degenerative effects of STZ. In case of Z. mays and S. cumini treated groups showed mild fatty degeneration and atrophy of beta cells in the pancreatic acinar cells. (Figure 5).[21]

STZ- induced diabetic group showed enhanced gene expression of iNOS, which was found to be diminished in the groups which received natural extracts of *A. heterophyllus*, *Z. mays*, *S. cumini* and Shilajit. (Figure 6).[12]

CONCLUSION

Anti-oxidative, hypoglycemic and hypolipidemic efficiency of *A.heterophyllus, Z .mays* silk, *S. cumini* and Shilajit were demonstrated in the present study. The biological mechanisms by which natural products exerts their effects are not well understood. The composition of fiber, phenolics, flavonoid etc may govern the anti-diabetic effects independently or in synergy. Our results also reinforce the benefit of natural products as preventive and/or therapeutic agents in management of diabetes. Hence further research is needed to support and encourage use of natural products in managing diabetes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

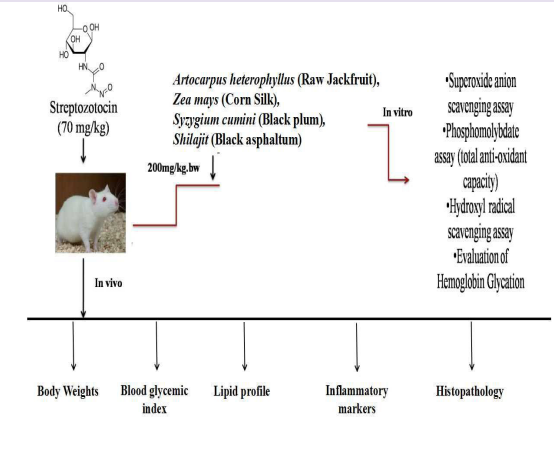
ABBREVIATIONS

STZ: Streptozotocin; **iNOS:** Inducible Nitric oxide synthase; **AGEs:** Advanced glycation end products.

REFERENCES

1. Lebovitz HE. Diagnosis, classification, and pathogenesis of diabetes mellitus. J Clin Psychiatry. 2001;62:5-9,40-1.
2. Meeprom A, Sompon W, Chan CB, Adisakwattana S. Isoferulic acid, a new anti-glycation agent, inhibits fructose- and glucose-mediated protein glycation *in vitro*. Molecules. 2013;186:6439-54.
3. Perera HK, Handuwalage Z, Mays silk. Analysis of glycation induced protein cross-linking inhibitory effects of some antidiabetic plants and spices. BMC. Complement Altern Med. 2015;15:175.
4. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;1079:1058-70.
5. Rizvi SI, Mishr N. Traditional Indian medicines used for the management of diabetes mellitus. Jour Diabetes. 2013;2013:1-11.
6. Rodrigues E, Mariutti LRB, Faria AF, Mercadante AZ. Microcapsules containing antioxidant molecules as scavengers of reactive oxygen and nitrogen species. Food. Chem. 2012;134:704-11.
7. Jayaprasad B, Sharavanan PS, Sivaraj R. Antidiabetic effect of *Chloroxylon swietenia* bark extracts on streptozotocin induced diabetic rats. Beni-Suef University Journal of Basic and Applied Sciences. 2016;5 (1):61-69.
8. Poolsil P, Promprom W, Talubmook C. Anti-hyperglycemic and Anti-hyperlipidemic Effects of Extract from *Houttuynia cordata Thumb*. In Streptozotocin-Induced Diabetic Rats. Pharmacognosy Journal. 2017;9 (3):382-7.
9. Gupta R, Gupta R. Hypolipidemic activity of *Pterocarpus marsupium* in Streptozotocin induced diabetes. J Complement Integr Med. 2009;6:1-28.
10. Rotimi SO, Omotosho OE, Rotimi OA. Persistence of acidosis in alloxan-induced diabetic rats treated with the juice of *Asystasia gangetica* leaves. Pharmacognosy Magazine. 2011;7 (25):25-30.
11. Ibegbulem CO, Chikezie PC. Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium, Aloe perryi, Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (Rattus norvegicus). Pharmacog Commun. 2013;3 (2):12-6.
12. Rabinovitch A, Suarez-Pinzon W, Sorensen O, Bleackley RC. Inducible nitric oxide synthase (iNOS) in pancreatic islets of non-obese diabetic mice: identification of iNOS expressing cells and relationships to cytokines expressed in the islets. Endocrinology. 1996;137:2093-9.
13. Nyenwe EA, Jerkins TW, Umpierrez GE, Kitabchi AE. Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes. Metabolism. 2010;601:1-23.
14. Chen J, Mangelinckx S, Adams A, Wang ZT, Li WL, De Kimpe N. Natural flavonoids as potential herbal medication for the treatment of diabetes mellitus and its complications. Nat. Prod. Commun. 2015;2101:187-200.
15. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: pharmacological and toxicological considerations. J Ethnopharmacol. 2014;155 (2):857-924.
16. Stanojević L, Stanković M, Nikolić V, Nikolić L, Ristić D, Čanadanovic-Brunet J *et al.* Antioxidant Activity and Total Phenolic and Flavonoid Contents of *Hieracium pilosella L*. Extracts. Sensors (Basel). 2009;97:5702-14.
17. Omar HS, El Beshbishy HA, Moussa Z, Taha KF, Singab AN. Antioxidant activity of *Artocarpus heterophyllus* Lam. (Jack Fruit) leaf extracts: remarkable attenuations of hyperglycemia and hyperlipidemia in streptozotocin-diabetic rats. Scientific World Journal. 2011;11:788-800.
18. Guo J, Liu T, Han L, Liu Y. The effects of corn silk on glycaemic metabolism. Nutr. Metab (Lond). 2009;6:47.
19. Srivastava S, Chandra D. Pharmacological potentials of *Syzygium cumini:* a review. J Sci Food Agric. 2013;939:2084-93.
20. Nair RB, Santhakumari G. Anti - diabetic activity of the seed kernel of *Syzygium cumini linn*. Anc Sci Life. 1986;62:80-4.
21. Bhattacharya SK. Shilajit attenuates Streptozotocin induced diabetes mellitus and decrease in pancreatic islet superoxide dismutase activity in rats. Phytotherapy Research. 1995;9 (1):41-4.
22. Ravishankara MN, Shrivastava N, Padh H, Rajani ML. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus R. Br.* (Anantmul). Phytomedicine. 2002;9 (2)153-60.
23. Umamaheswari U, Chatterjee TK. *In vitro* antioxidant activities of the fractions of *Coccinia grandis L*. leaf extract. Afr J Tradit Complement Altern Med. 2008;5 (1):61-73.
24. Kwon DY, Kim SJ, Lee LW, Kim YC. Comparison of hydroxyl radical, peroxyl radical, and peroxynitrite scavenging capacity of extracts and active components from selected medicinal plants. Toxicol Res. 2010;26 (4):321-7.
25. Adisa RA, James O, Satar AO, Olufunso O. Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extracts of *Cnestis ferruginea*. Journal of the Cameroon Academy of Sciences. 2004;4 (3):351-9.
26. Asaduzzaman M, Nahar L, Rahman MS, Hasan M, Khatun A. Hypoglycemic and Hypolipidemic Potential of *Nigella sativa L*. Seed Extract in Streptozotocin (STZ)-Induced Diabetic Rats. J Plant Biochem Physiology. 2015; I3:158.
27. Alphosus OO, Chizaramok OA. Renal and Hepatic Antioxidant Status of Hyperglycemic Rats Treated With Single and Combinatorial Herbal Formulations. Pharmacognosy Communications. 2015;5 (2):148-59.
28. Jagdish K, Mehul S, Nehal S. Effect of Hesperidin on Serum Glucose, HbA1c and Oxidative Stress in Myocardial Tissue in Experimentally Induced Myocardial Infarction in Diabetic Rats. Pharmacognosy Journal. 2010;2 (7):185-9.

GRAPHICAL ABSTRACT



SUMMARY

- The present study reports the anti-oxidative, hypoglycemic and hypolipidemic efficiency of *A.heterophyllus*, *Z .mays* (Corn silk), *S. cumini and Shilajit* in both *in vitro* and *in vivo* studies.
- The ethanolic extracts of *A.heterophyllus, Z .mays* (Corn silk), *S. cumini and Shilajit* has not shown any side effects.
- The efficiency of these ethanolic extracts was evenly matched to the efficiency of commercially available anti-diabetic drugs, hence these extracts can be used as therapeutic agents in management of dia¬betes and these are cost effective as well.

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