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RESEARCH ARTICLE

Evaluation of Antidiabetic Potential of New Dosage Forms of AYUSH 82 in Streptozotocin-induced Type II Diabetic Rats

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ABSTRACT

Introduction: AYUSH 82 powder is an Ayurvedic antidiabetic formulation developed by the Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India. The antidiabetic potential of AYUSH 82 powder along with its two new dosage forms—AYUSH 82 mixture extract and AYUSH 82 compound extract—has been established to be mediated via α-amylase inhibitory property, insulin-dependent glucose uptake in skeletal muscle cell line (C2C12 myotubes).

Aim: In the current study, we investigated the *in vivo* antidiabetic potential of new dosage forms of AYUSH 82—AYUSH 82 mixture extract and AYUSH 82 mixture extract + Shilajit.

Results and conclusion: Oral administration of AYUSH 82 mixture extract for 28 days twice daily could significantly increase serum insulin levels, reduction in Glucose Area under Curve 0 to 90 minutes, fed blood glucose, and triglyceride levels. It also showed improvement in histopathological grading, which resulted in protecting the pancreatic islets from progressive damage. Addition of Shilajit to AYUSH 82 mixture extract did not contribute much improvement in biochemical parameters related to diabetes.

Keywords: AYUSH 82 mixture extract, Shilajit streptozotocin, Type II diabetes mellitus.

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INTRODUCTION

Type II diabetes mellitus (DM) is a chronic metabolic disorder whose global prevalence has been increasing steadily. As such, it is on the verge of becoming an epidemic in some countries. The number of people affected by DM is expected to double in the next decade, thereby adding to the already existing burden for health care providers, especially in poorly developed countries.[1] Diabetes mellitus is diagnosed based on sustained hyperglycemia. People with DM are at elevated risk for a number of serious health problems, including cardiovascular disease, premature death, blindness, kidney failure, amputations, fractures, frailty, depression, and cognitive decline.[2] AYUSH 82 is an Ayurvedic formulation for diabetes developed by the CCRAS, Ministry of AYUSH, Government of India. It comprises ingredients traditionally used for their beneficial effect in diabetes (*Prameha/Madhumeha*).[3] Glucose-lowering effects of AYUSH 82 powder have been reported in patients of *Madhumeha* where AYUSH 82 powder (5 gm TDS with 500 mg of pure Shilajit BD for 24 weeks) was found to significantly reduce fasting and postprandial blood sugar levels along with clinical improvement in diabetic subjects.[4] In another clinical study, AYUSH 82 administered at similar doses to patients of noninsulin-dependent DM was found to produce significant reduction ($p > 0.001$) in both fasting and postprandial blood sugar and also showed marked improvement in symptoms of diabetes like polyuria, polydipsia, polyphagia, weakness, and exhaustion after a treatment period of 6 weeks.[5] The antidiabetic potential of AYUSH 82 powder along with its two new dosage forms—mixture extract and compound extract when investigated *in vitro* through mechanism of their action exhibited inhibition of α-amylase activity and an increase in insulin-dependent glucose uptake in C2C12 myotubes as compared with control.[6] In the current study, we investigated the *in vivo* antidiabetic potential of AYUSH 82 mixture in comparison to AYUSH 82 mixture extract with Shilajit. The study was conducted in streptozotocin (STZ)-induced type II diabetic rat model.

MATERIALS AND METHODS

Chemicals/Reagent/Media

Streptozotocin (Sigma), metformin hydrochloride I.P-500 mg (Cipla), Mercer Expert Assay rat insulin enzyme-linked immunosorbent assay (ELISA) kit.

Preparation of AYUSH 82 Mixture Extract and AYUSH 82 Mixture Extract + Shuddh Shilajit

For AYUSH 82 mixture extract (DRDC/2016/008): Hydroalcoholic extraction of equal quantities of individual herbs (Karavellaka, Jamun, Amra, and Gurmar) was done and then mixed together. The extract was concentrated and dried to fine powder, which was checked for compliance with specification parameters (Table 1).

For AYUSH 82 mixture extract + Shuddh Shilajit (DRDC/2016/009): Hydroalcoholic extraction of equal quantities of individual herbs (Karavellaka, Jamun, Amra, and Gurmar) were taken and then mixed together along with Shuddh Shilajit. The extract was concentrated and dried to fine powder, which was checked for compliance with specification parameters.

Experimental Animals

Male Sprague Dawley rats (3–5 days old) weighing 7 to 10 gm were housed under standard laboratory conditions of target room temperature: 22 ± 3°C, target relative humidity: 30 to 70%, light/dark cycle: 12-hourly, feed: conventional feed purchased from a commercial supplier were provided in fixed amount to the animals. Water: filtered drinking water *ad libitum*. The experimental procedures were conducted after the approval of Ethical Committee—CPCSEA, dated April 18, 2016 with approval No. IAEC/37/422, Ministry of Environment and Forest, Government of India.

Antidiabetic Activity

STZ-induced Sprague Dawley Rat Model for DM

Preparation of STZ Solution: Aliquots of STZ (Sigma) 10 mg were weighed and covered with aluminum foils to protect from light. The STZ aliquots were dissolved in freshly prepared ice cold 0.1 M citrate buffer, pH 4.5, and were used within 15 minutes.

Induction of Diabetes: Male Sprague Dawley pups (n = 40) were administered with intraperitoneal injection of a freshly prepared STZ at the dose of 80 mg/kg in an ice-cold 0.1 M citrate buffer (pH 4.5). Rat pups (n = 8) in nondiabetic control group were administered with citrate buffer.

Table 1: Composition details of AYUSH 82 (each 100 gm)

Sanskrit name	*Botanical name*	*Part used*	*Qty (gm)*
Karavellaka	*Momordica charantia* L.	Dried fruit	25
Jambu	*Syzygium cumini* (L.) Skeels	Seed	25
Amra	*Mangifera indica* L.	Seed	25
Gurmar	*Gymnema sylvestre* R.Br.	Leaves	25

Experimental Design

Randomization: After weaning off STZ injection (day 21), the animals were kept in groups of five in collective cages, with a full access to food and water for the following 8 weeks.

Eight weeks postinjection, diabetic animals were randomized into four groups containing eight animals in each group based on the oral glucose tolerance test (OGTT) which was carried out at 0, 30, and 90 minutes. Based on insulin resistance, the animals were selected and grouped. For OGTT, animals were fasted for 16 hours. Basal blood glucose levels (BGL) were estimated at 0 minute. All the animals including those in normal control group were administered orally with glucose at the dose of 2 gm/kg by gavage. The BGL were estimated at 30 and 90 minutes, postglucose administration. The BGL values at various time points were subjected for GAUC 0 to 90 calculation by WinNonlin software. Based on the results of OGTT, randomization was done in which 32 animals were divided into four groups, each consisting of eight animals as follows:

- Group I: Served as normal control and received vehicle [0.5% Carboxymethyl cellulose (CMC)] at the dose volume of 10 mL/kg b.i.d. for 28 days.
- Group II: Served as diabetic control and received vehicle (0.5% CMC) at the dose volume of 10 mL/kg b.i.d. for 28 days.
- Group III: Served as STZ-induced diabetic rats treated with AYUSH 82 mixture extract orally at the dose of 103 mg/kg b.i.d. for 28 days.
- *Group IV*: Served as STZ-induced diabetic rats treated with AYUSH 82 mixture extract + Shuddh Shilajit orally at the dose of 154 mg/kg b.i.d. for 28 days. AYUSH 82 mixture extract and Shilajit were in the proportion of 2:1, i.e., 33.69% of Shilajit in total formulation.
- Group V: Served as STZ-induced diabetic rats treated with standard reference drug, i.e., metformin at the dose of 250 mg/kg b.i.d. for 28 days.

Dosing: Dosing was initiated on day 1 evening and finished on day 29 morning to complete the 28 days twice-daily dosing regimen. Body weight, feed intake, and clinical signs were measured daily throughout the study period. BGL was measured weekly. On day 29, OGTT was repeated. At the end of the treatment period (day 29), blood was collected from rats by retro-orbital puncture and centrifuged. Serum was separated for estimation of glucose and lipid profile (triglyceride and cholesterol).

Serum insulin also was estimated using commercially available ELISA kit. Thereafter, animals were humanely sacrificed; pancreas was collected, weighed, and preserved in 10% buffered formalin for histopathological (hematoxylin and eosin staining) investigation.

RESULTS

Clinical Signs and Observation

Daily cage-side observations were made to detect clinical signs and mortality from the day of dosing till end of the study. No clinical signs of toxicity were found during experimental period.

Body Weight

Body weight of all the animals was recorded from day 1 to day 28. The percent change in body weight for each animal was calculated. Decreasing trend in percent body weight change was observed in diabetic control rats from day 7 to 28 as compared with normal control. No significant difference in percent bodyweight change was observed among the treatment groups as compared with diabetic control.

Feed Intake

During the study (d2–28), the mean of cumulative feed consumption was lower in disease control as compared with normal control. However, no significant difference in cumulative feed consumption was observed among the treatment groups as compared with diabetic control.

Blood Glucose

Fed BGLs were measured weekly (day 1, 7, 14, 21, 28, and 29) by using Glucose Meter Contours™ TS. Mean fed BGL and percent change in blood glucose with respect to diabetic control was calculated for each group.

Mean Fed

There was significant increase in BGL for (G2) diabetic control animals, which was 145.35 mg/dL as compared with normal control (G1) animals whose mean BGL was 109.36 mg/dL on day 29. This increase in fed BGL in diabetic control animals (G2) was observed throughout the study. AYUSH 82 mixture extract (G3) treatment showed 10.69% reduction in fed BGL on day 29 when compared with diabetic control (G2). Animals treated with AYUSH 82 mixture extract + Shuddh Shilajit showed 5.62% reduction on day 29. Metformin 250 mg/kg-treated rats have shown 4.72% reduction in fed BGL on day 29. However, there was no statistical significant difference between the groups (Graph 1).

Glucose Tolerance Test

Significant increase ($p < 0.001$) in $GAUC_{0–90}$ level was observed in disease control when compared with normal control. The $GAUC_{0–90}$ level of AYUSH 82 mixture extract (G3)-treated group was 14375.63 mg/dL but the $GAUC_{0–90}$ level of diabetic control group was 16,284.38 mg/dL. AYUSH 82 mixture extract (G3) group had considerable effect; 11.72% reduction in mean $GAUC_{0–90}$ level as compared with diabetic control. AYUSH 82 mixture extract + Shuddh Shilajit (G4) group animals did not show any effect in reducing the $GAUC_{0–90}$ and the mean value was 16655.63 mg/dL, which was 2.28% more than diabetic control. Mean $GAUC_{0–90}$ level of 13,325.63 mg/dL was observed in metformin-treated rats (G5), which showed 18.17% reduction in OGTT as compared with diabetic control on day 29 (Graph 2).

Biochemical Parameters

Increase in triglyceride level and decrease in cholesterol level were observed in disease control compared with normal control. The triglyceride levels in AYUSH 82 mixture extract (G3)-treated animals were 23.45% lower than those in diabetic control. AYUSH 82 mixture + Shuddh Shilajit-treated animals showed 10.87 and 11.26% decrease in cholesterol and triglycerides level respectively. In metformin-treated rats (G5), 22.52% reduction in mean triglyceride level and 6.17% reduction in mean cholesterol levels were observed compared with diabetic control (Graphs 3 and 4).

Insulin Levels

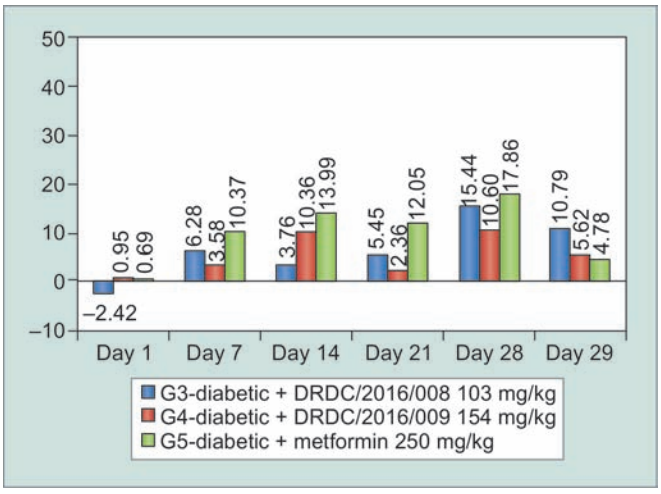
In AYUSH 82 mixture extract (G3)-treated rats, 25.83% increase in insulin level was observed as compared with diabetic control, which is statistically significant. AYUSH 82 mixture extract + Shuddh Shilajit treatment (G4) resulted in 9.61% increase in insulin level and metformin 250 mg/kg (G5)-treated rats showed 8.31% increase in insulin levels compared with diabetic control (G2) group animals (Graph 5).

Pancreas Weight

Decrease in pancreas weight was observed in disease control as compared with normal control. No significant difference in relative pancreas weight was observed among the treatment groups as compared with diabetic control.

Histopathology

On day 29, the rats were euthanized and the pancreas from all rats was collected for histology study. Mean



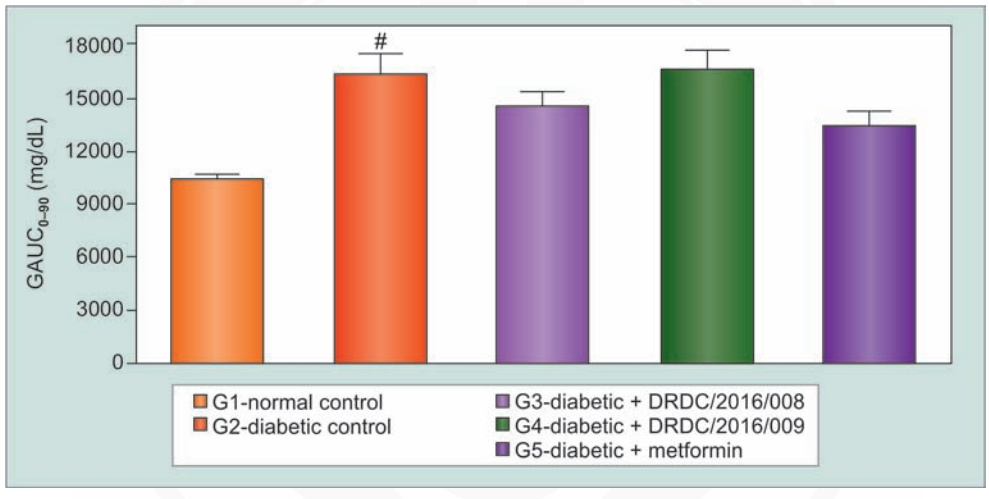
Graph 1: Percentage change in fed BGL

of reduced number and size of islets of Langerhans, decreased cellularity in islets of Langerhans, and degeneration of pancreatic cells were measured. The rats from diabetic control group showed a significantly ($p < 0.001$) reduced mean β-cell number, size, and markedly degenerated pancreatic islets as compared with normal control.

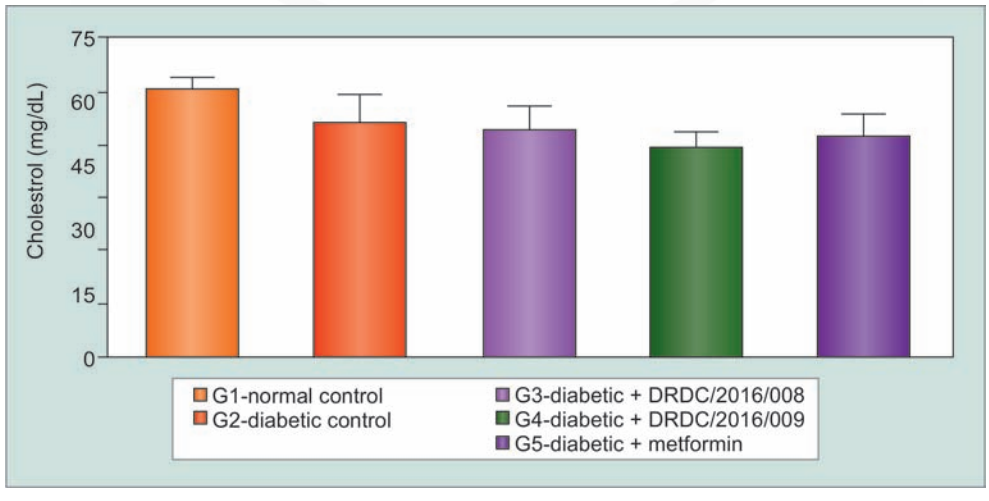
AYUSH 82 mixture extract (G3) group had marginal effect, i.e., 9.9% improvement in histopathological grading of various histological parameters as compared with diabetic control, which indicated tendency of protecting the islets from progressive damage due to STZ during the 28-day study period. However, 8.3% improvement in histopathological grading was observed in AYUSH 82 mixture extract + Shuddh Shilajit (G4) group animals. Metformin-treated rats showed 13.2% improvement in severity of histopathological lesions (Fig. 1).

DISCUSSION

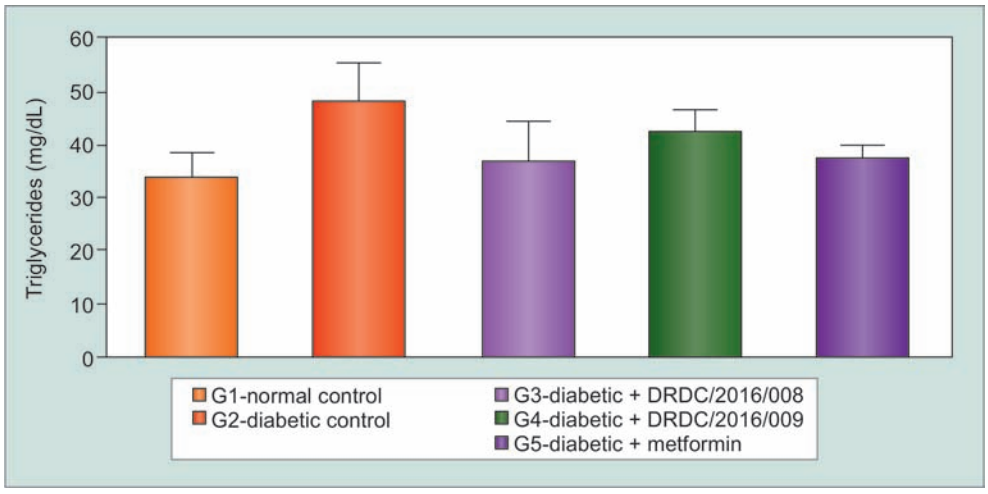
In vitro and *in vivo* study results suggested that AYUSH 82 mixture extract has antidiabetic potential which may be due to the ingredients present in the formulation like Jambu, Karavellaka, Amra, and Gurmar. The published literature available also supports the antidiabetic action



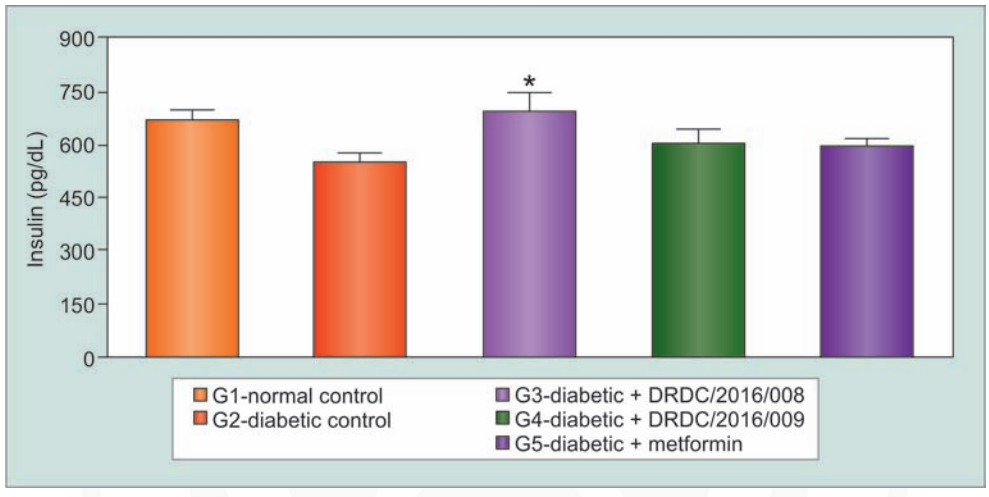
Graph 2: $GAUC_{0–90}$ day 29
#$p < 0.001$; one way ANOVA followed by Dunnett's test *vs* normal control



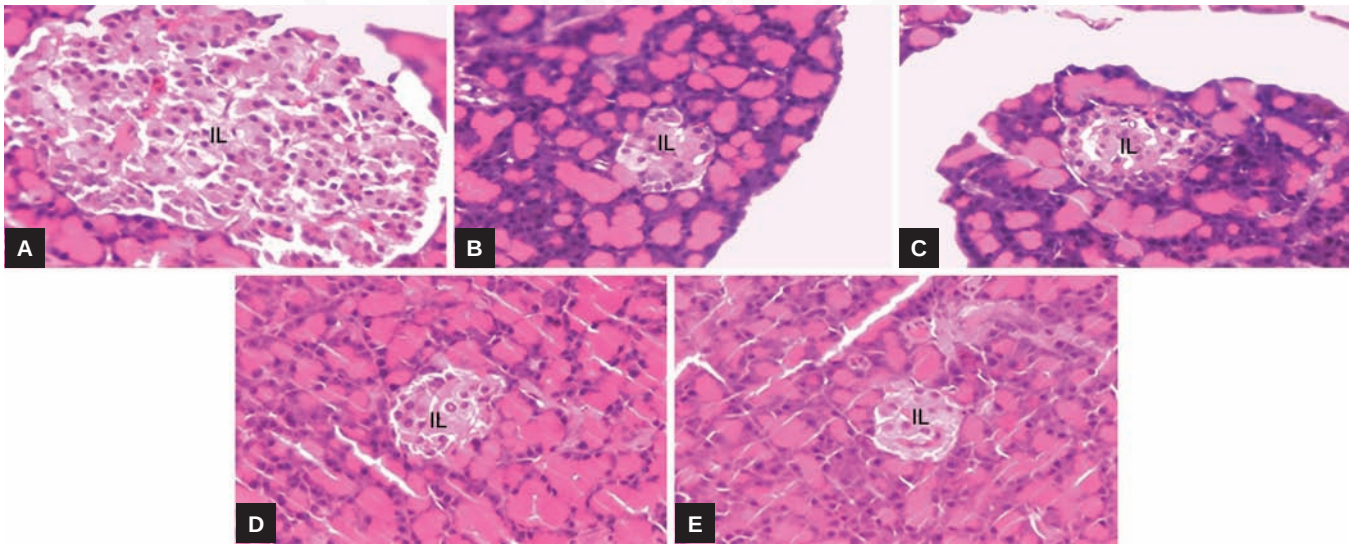
Graph 3: Mean cholesterol levels



Graph 4: Mean triglyceride levels



Graph 5: Effect of treatment on insulin levels
One way ANOVA diabetic control *vs* other treatment groups; *p < 0.05



Figs 1A to E: Representative histopathological section (H & E ×400) of pancreas of (A) vehicle control, (B) diabetic control, (C) diabetic + AYUSH 82 mixture extract, (D) diabetic + AYUSH 82 mixture extract and shilajit, (E) diabetic + metformin. Vehicle-treated group is showing normal structure of Islets of langerhans (IL), diabetic rat pancreas showing reduced mean β-cell number, size and markedly degenerated pancreatic islets. AYUSH 82 mixture extract and AYUSH 82 mixture extract and shilajit-treated groups are showing marginal improvement of Islets of langerhans. Diabetic rat treated with metformin showing improvement of Islets of langerhans

of the individual herbs of the formulation. In one of the published studies, the effects of powder and ethanol extract of *S. cumini* (Jambu) seeds (1.25 per kg body weight) treatment for 21 days on glucose homeostasis, serum insulin, serum lipids, and liver glycogen content in STZ-induced type II diabetic rats were evaluated. The administration of *S. cumini* seed powder and ethanol extract for 21 days to type II diabetic rats significantly reduced the fasting glucose level, although it did not alter the BGL after glucose challenge. The insulin level and liver glycogen content were also not changed after dietary administration of *S. cumini* seed powder or ethanol extract. In addition to hypoglycemic effect, the *S. cumini* significantly ameliorated the lipid profile. The plasma low-density lipoprotein-cholesterol level, an atherogenic lipid, significantly ($p < 0.01$) decreased with a concurrent increase ($p < 0.01$) in the plasma high-density lipoprotein-cholesterol level, thus suggesting dietary *S. cumini* could be used as one of the alternatives in the treatment of diabetes.[7] Methanol extract of *G. sylvestre* (Gurmar) leaf and callus showed antidiabetic activities through regenerating β-cells in treated rats, when compared with the standard diabetic rats.[8] Dihydroxy gymnemic triacetate from *G. sylvestre* at 20 mg dose produced significant effects on all biochemical parameters studied compared with diabetic control group.[9] The treatment of diabetic rats with *M. charantia* (Karavellaka) fruit extract over a 10-week period returned these levels close to normal. In addition, *M. charantia* juice also exhibited an inhibitory effect on membrane lipid peroxidation under *in vitro* conditions. These results suggest that *M. charantia* fruit extract exhibits hypolipidemic as well as hypoglycemic effects in the STZ-induced diabetic rat.[10] The alcoholic extract of *M. indica* (Amra) leaves and kernel seeds are reported to possess significant antidiabetic effect against alloxan-induced diabetes in Wistar rats and its stimulating insulin production in pancreas of Wistar rats.[11] Previously, *in vitro* studies on the various tested dosage forms of AYUSH 82 powder, mixture extract, and the compound extract have shown antidiabetic activity mediated either through inhibition of α-amylase activity or regulation of insulin -dependent glucose uptake in mouse skeletal cell line.[6]

CONCLUSION

Oral administration of AYUSH 82 mixture extract twice daily for 28 days to STZ-induced Sprague Dawley rats showed significant increase in serum insulin levels, reduction in $GAUC_{0-90}$, fed BGL, and triglyceride levels. It also showed improvement in histopathological grading, which resulted in protecting the pancreatic islets from progressive damage.

No statistical difference was observed in any of the treated groups (AYUSH 82 mixture extract, AYUSH 82 mixture extract + Shilajit or metformin group) in any of the biochemical parameters related to diabetes. However, statistically significant improvement in parameters related to diabetes was observed in treated groups as compared with untreated control diabetic rats.

Based on the above results, it can be concluded that AYUSH 82 mixture extract has antidiabetic potential. Addition of Shilajit to AYUSH 82 mixture extract did not contribute to much improvement in biochemical parameters related to diabetes.

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हिन्दी सारांश

स्ट्रेप्टोजोटोसिन प्रेरित टाइप II डायबेटिक रेट्स में आयुष 82 की नई औषध मात्रा की मधुमेहरोधी क्षमता का मूल्यांकन

पृष्ठभूमिः आयुष 82 चूर्ण केन्द्रीय आयुर्वेदीय विज्ञान अनुसंधान परिषद् (सीसीआरएएस), आयुष मंत्रालय, भारत सरकार द्वारा विकसित एक आयुर्वेदिक मधुमेहरोधी औषध योग है। आयुष–82 यौगिक सार की आयुष–82 चूर्ण की इसकी दो नई औषध मात्राओं– आयुष 82 मिश्रण सार एवं आयुष–82 यौगिक सार की मधुमेहरोधी क्षमता ए–अमाइलेज अवरोधक गुण व स्केलेटल मांसपेशी कोशिका लाइन (सी2सी 12 मायोटयूब्स) के माध्यम से स्थापित किया गया है।

उद्देश्यः वर्तमान अध्ययन में हमने आयुष–82 की नई औषध मात्राओं आयुष–82 मिश्रण सार एवं आयुष–82 मिश्रण सार + शिलाजीत की इन वाईवो मधुमेहरोधी क्षमता की जाँच की।

परिणाम एवं निष्कर्षः आयुष–82 मिश्रण सार का दिन में दो बार 28 दिनों तक मुख द्वारा प्रयोग करने पर सीरम इन्सुलिन स्तर में महत्त्वपूर्ण वृद्धि, 0 से 90 मिनट्स कर्व के अन्तर्गत शर्करा क्षेत्र में कमी, रक्तशर्करा स्तर व ट्राइग्लिसराइड स्तर में कमी पाई गई। ऊतक विकृति विज्ञानीय ग्रेडिंग में भी सुधार देखा गया, जिसके परिणाम स्वरुप अग्न्याशयी आइस्लेट्स को प्रगतिशील क्षति में बचाया गया। आयुष–82 मिश्रण सार में शिलाजीत के संयोजन से मधुमेह संबंधी जैवरासायनिक मापदण्डों में अधिक सुधार नहीं हुआ।

मुख्य शब्दः आयुष–82 मिश्रण सार, शिलाजीत, स्ट्रेप्टोजोटोसिन, टाइप II डायबिटीज मेलाइटस