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EXPLORATION OF ANTI-HYPERGLYCEMIC POTENTIAL OF HERBAL MIXTURE IN STREPTOZOTOCIN-INDUCED TYPE-II DIABETES IN RATS

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Keywords:

Herbal mixture, Streptozotocin, *Withania coagulans*, *Alstonia scholaris*, *Aegle marmelose*

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ABSTRACT: Indigenous plants like *Withania coagulans* fruits. *Alstonia scholaris* bark and *Aegle marmelose* leaves have been used for diabetes management by traditional Indian people for a long time. The present study was carried out to investigate anti-hyperglycemic potential of an herbal mixture comprising of extracts of these three plants in streptozotocin-induced diabetes in Wistar rats and to focus on its possible mechanism of action. Experimental diabetes was induced in Wistar rats by a single intraperitoneal injection of streptozotocin (65 mg/kg). Animals were divided in five groups (n=6) and treated with herbal mixture (100 & 200 mg/kg) for 4 weeks. At the end of the study period, fasting blood glucose, oral glucose tolerance, glycolsylated hemoglobin, serum insulin, glucose uptake from rat hemidiaphragms, and liver glycogen were determined. Antioxidant enzymes of the liver, including superoxide dismutase and catalase, were evaluated. Histopathology of the pancreas was studied. The results of our study demonstrate anti-hyperglycemic and antioxidant potential of the herbal mixture, justifying its use in the indigenous system of medicine.

INTRODUCTION: Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Lack of insulin affects the metabolism of carbohydrates, protein, and fat, leading to significant water and electrolyte homeostasis disturbance. The long-standing metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, with those of the vascular system being particularly susceptible.

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These changes lead to the development of well-defined clinical entities, the so-called 'complications of diabetes' which characteristically affect the eye, kidney and nervous system [1-2]. The aging population, consumption of calorie-rich diet, obesity, and sedentary lifestyle has led to a tremendous increase in the number of diabetic individuals worldwide.

It has been estimated that India, considered the diabetic capital of the world with more than 32 million diabetic patients, would continue to lead even in 2030 with a whopping 80 million diabetics [3-4]. The enormous cost of modern treatment stressed to the evolution of alternative strategies for the prevention and treatment of diabetes. Although insulin has become one of the most important therapeutic agents known to medicine, there is continuing effort to find insulin substitutes,

secretagogues, or sensitizers from synthetic or plant sources for the treatment of diabetes. Almost 90% of the people in developing countries still rely on traditional medicines and this has led to the discovery of some new drugs now in professional use worldwide [5]. Moreover, herbal remedies are apparently effective, produce minimal or no side effects in clinical experience and are of relatively low costs as compared to oral synthetic hypo-glycemic agents. Considering this need of alternative therapy in the management of diabetes mellitus, we have evaluated anti-hyperglycemic activity of indigenous plants, including *Withania coagulans* fruits, *Alstonia scholaris* bark and *Aegle marmelose* leaves in our earlier studies [6-8]. Based on those plants studied, we have undertaken the present study to explore the antidiabetic potential of an herbal mixture containing these three plant extracts.

MATERIALS AND METHODS:

Plant Materials: The leaves of *Aegle marmelose* were collected from Nigdi, Pune in the month of January 2017. Bark of *Alstonia scholaris* was procured from Mankarnika Ayurvedic Bhandar, Pune. Fruits of *Withania coagulans* were collected from hilly regions of Trimbakeshwar, Nasik in month of March 2017. Dr. A. M. Mujumdar, Head, Plant Sciences Division, Agharkar Research Institute, Pune, authentified the leaves of Aegle marmelose (Auth. 17-21), the bark of *Alstonia scholaris* (Auth.17-64) and Fruits of *Withania coagulans* (Auth. 17-45). The plant materials were dried in the shade, ground to a coarse powder by using a dry grinder, and stored in an air-tight container for further use.

Preparation of Extracts: The aqueous extract of *Alstonia scholaris* bark (AEAS) and *Withania coagulans* fruits (AEWC) were prepared by the method of maceration. 250 g powdered material was put in 1000 ml of water for 48 h with intermittent stirring. It was filtered, the supernatant was collected and evaporated to dryness under reduced pressure in a rotary evaporator. Methanolic extract of *Aegle marmelose* leaves (MEAM) was prepared by the method of Soxhlet extraction. The powedered leaves of *Aegle marmelose* were extracted with methanol at 55 ºC-60 ºC using a Soxhlet extractor. The process of extraction was repeated to get a sufficient quantity. The methanol was evaporated at 70 ºC. The extracts were dried by evaporation under reduced pressure using rotavapor bath.

Preparation of Herbal Mixture: Herbal Mixture was prepared by mixing 2:3:1 parts of methanolic extract of *Aegle marmelose* leaves, aqueous extract of *Alstonia scholaris* bark, and aqueous extract of *Withania coagulans* fruits. Different concentrations of the herbal mixture were prepared freshly everyday using carboxymethyl cellulose for the period of 28 days.

Acute Toxicity Study: Acute toxicity study was performed according to OECD guidelines no 423. Wistar rats selected by random sampling technique were employed in this study. The animals were fasted overnight with free access to water. Herbal mixture was administered orally to different groups at increasing dose levels of 50, 100, 300, 2000, and 5000 mg/kg body weight. After dosing, the animals were observed for 2 h and then intermittently for a further 4 h for behavioral changes and finally recording mortality up to 24 h till 14 days [9].

Animals: Wistar rats weighing between 180-220 g were used for the study. The rats were procured from Yash Farms, Pune. They were caged in standard polypropylene cages of size 38 × 33 × 10 cm with stainless steel coverlids. The animals were fed on a standard pellet diet (Pranav Agro Industries, Pune, India) and water was freely available.

The animals were maintained in a controlled environment (12-h light/dark cycle) and temperature (30 ±2 ºC). Before every experiment, rats were kept fasting for 18-24 h and water was allowed *ad libitum*. All the experimental procedures are carried out in accordance with IAEC as per the guidelines of CPCSEA (Reg. No. 884/ac/05/CPCSEA). The preparations (extracts, standard drugs, and vehicles) were administered orally with a feeding needle and syringe directly into the stomach.

Drugs and Chemicals: Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glibenclamide was procured from Aventis Pharma Ltd (India). All other chemicals were procured from local sources and were of analytical grade.

Induction of Diabetes: Streptozotocin was dissolved in ice cold citrophosphate buffer (pH 4.3) immediately before use and was administered intraperitoneally (65 mg/kg) to overnight fasted rats. In order to avoid the STZ-induced hypo-glycemic mortality, 5% glucose solution was given for 24 h to STZ- treated animals. The fasting blood glucose was estimated after 72 h of STZ adminis-tration to confirm the diabetic state. Rats showing fasting blood glucose more than 200 mg/dl were considered diabetic and used for study [10].

Experimental Groups:

Group I: Normal control where rats received CMC suspension (0.1%) for 28 days.

Group II: Diabetic control where rats received single dose of STZ intraperitoneally (65 mg/kg).

Group III: Diabetic rats received 100 mg/kg herbal mixture orally for 28 days.

Group IV: Diabetic rats received 200 mg/kg herbal mixture orally for 28 days.

Group V: Diabetic rats received glibenclamide (4 mg/kg).

Evaluation Parameters:

Fasting Blood Glucose: Fasting blood glucose was determined on 0, 7^{th}, 14^{th}, 21^{st}, and 28^{th} day of the study period using a glucometer (Accu Check, Germany).

Oral Glucose Tolerance Test: Oral glucose tolerance test (OGTT) was performed in overnight fasted (18 h) diabetic rats at the end of 28th day of study. Glucose (3 g/kg) was orally given 30 min after the administration of herbal mixture or standard drug. Blood glucose was determined at 0, 30, 60, 90 and 120 min of glucose administration [11].

Glycosylated Hemoglobin: Glycosylated hemo-globin was determined in heparinised whole blood by ion exchange resin method [12].

Plasma Insulin: Insulin concentrations were determined through a radio immunoassay procedure, using insulin kit (Coral, India) according to manufacturer's instructions.

Estimation of Glucose Uptake by Isolated Rat Diaphragm: It is performed by the method described by Chattopadhyay *et al.,* [13] The hemidiaphragms of rats were placed in two small tubes containing 2 ml of tyrode solution with 2% glucose and incubated for 30 min at 37º ± 0.2 ºC with appropriate aeration. Glucose uptake by the hemidiaphragm was calculated as the difference between the initial and final glucose content in the incubation medium [14].

Liver Glycogen Estimation: Liver of individual animal was homogenized in 5% w/v trichloroacetic acid and its glycogen content was estimated by the method of Carrol [15].

Estimation of Liver Superoxide Dismutase (SOD): Assay mixture includes 2.95 ml Tris-HCl buffer, 25 µl of pyrogallol, and 0.05 ml of tissue homogenate in the total volume of 3 ml. The difference between the optical densities obtained at 1.30 and 3.30 min was estimated and expressed as U/mg protein [16].

Estimation of Liver Catalase (CAT): The catalase activity in the liver homogenate was estimated using a quantitative spectrophotometric technique developed for routine studies of catalase kinetics following the breakdown of hydrogen peroxide into water and oxygen [17].

Histopathology of Pancreas: Pancreatic tissues were removed after sacrificing the animal. Small slices of tissue samples were fixed in 10% formalin for 4-5 h and then processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin (H&E) for histopathological study [18].

Statistical Analysis: The results are presented as Mean ± S.E.M of 6 rats per group. Data were analyzed using one-way analysis of variance followed by Dunnett's multiple comparison test for analysis of biochemical data using the software GraphPad Instat (Version-3). Values were considered statistically significant at $p<0.05$.

RESULTS: Diabetic control group showed increase in blood glucose level as compared to normal rats as shown in **Table 1**. Oral administration of herbal mixture (100 and 200 mg/kg) for 28 days showed a dose-dependent significant ($p<0.01$) decrease in fasting blood glucose in STZ diabetic rats.

TABLE 1: EFFECT OF HERBAL MIXTURE ON FASTING BLOOD GLUCOSE IN STZ- INDUCED DIABETES IN RATS

Experimental Groups	Fasting Blood Glucose (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control (NC)	75.5 ±3.69	73.5±2.23	72.16±1.81	75.33±2.23	75.16±2.16
Diabetic control (DC)	293.0 ±7.75[a]	295.5±6.8[a]	298.5±7.72[a]	293.66±7.78[a]	299.16±7.8[a]
DC+ Herbal mixture (100 mg/kg)	294.33 ±7.56	221.83 ±6.14[**]	204.33 ±6.46[**]	158.83 ±5.43[**]	130.83 ±5.71[**]
DC+ Herbal mixture (200 mg/kg)	296.16 ±7.27	218.5 ±6.56[**]	198.5±5.99[**]	140.66±4.5[**]	108.5 ±4.17[**]
DC+ GL (4 mg/kg)	297.66±7.86[**]	214.66 ±6.17[**]	190.83±6.5[**]	143±3.92[**]	120.33 ±4.17[**]

Values are expressed as mean ± S.E.M of six rats per group. NC: Normal control, DC: Diabetic control. GL: Glibenclamide. Data analyzed by ANOVA followed by Dunnett's multiple test for comparison. ap<0.01: Significant difference of diabetic control from normal control. **p<0.01, *p<0.05: Significant difference of treated groups from diabetic control on corresponding days

TABLE 2: EFFECT OF HERBAL MIXTURE ON ORAL GLUCOSE TOLERANCE IN GLUCOSE LOADED RATS

Experimental Groups	Fasting Blood Glucose (mg/dl)			
	0 min	30 min	60 min	120 min
Glucose Control (GC)	73.66±2.94	215.33 ±9.26	128.5±4.44	101.66±5.99
DC+Herbal mixture (100 mg/kg)	72.16±2.6	160.66±5.21[**]	104.16±5.21[**]	89.16±5.4
DC+Herbal mixture (200 mg/kg)	73.5±1.17	134.16±6.29[**]	96.33±4.27[**]	85.0±4.56[*]
DC+ GL (4 mg/kg)	74.5±3.19	115±2.74[**]	90.16±5.51[**]	80.83±5.1[**]

Values are expressed as mean ± S.E.M of six rats per group. GC: Glucose control. GL: Glibenclamide. Data analyzed by ANOVA followed by Dunnett's multiple test for comparison. **p<0.01, *p<0.05: Significant difference of treated groups from glucose control

As shown in **Table 2**, glucose control rats showed sharp rise in blood glucose level at 30 minutes after glucose load. Administration of herbal mixture (200 mg/kg) showed significant (p<0.05) reduction in glucose threshold, 30 min post-glucose loading in normal rats. Glibenclamide normalized the blood glucose within 60 min in glucose-loaded normal rats. Diabetic control group showed a significant decrease in liver glycogen, serum insulin, and glucose uptake by rat diaphragm, while there was a significant increase in glycosylated haemoglobin as compared to the normal control group, as shown in **Table 3**. Treatment of diabetic rats with the herbal mixture (100 and 200 mg/kg) for 28 days showed significant (p<0.05) improvement in liver glycogen, serum insulin, and glucose uptake by rat diaphragm, while there was significant (p<0.05) decrease in glycosylated hemoglobin.

TABLE 3: EFFECT OF HERBAL MIXTURE ON BIOCHEMICAL PARAMETERS IN STZ INDUCED DIABETES IN RATS

Experimental Groups	Liver glycogen (g/100g)	Glycosylated Hb (%)	Serum insulin (µIU/ml)	Glucose up-take by diaphragm (mg/100g)
Normal control (NC)	3.5±0.1	5.38±0.21	3.4±0.04	12.35±0.39
Diabetic control (DC)	0.83±0.01[a]	8.53±0.2[a]	1.9±0.04[a]	5.46±0.43[a]
DC+ Herbal mixture (100 mg/kg)	1.9±0.07[*]	7.24±0.11[*]	2.4±0.08[**]	8.68±0.66[**]
DC+ Herbal mixture (200 mg/kg)	2.46±0.11[**]	6.91±0.21[**]	2.7±0.04[**]	10.4±0.21[**]
DC+ GL (4 mg/kg)	3.1±0.1[**]	6.13±0.25[**]	2.9±0.03[**]	12.6±0.31[**]

Values are expressed as mean ± S.E.M of six rats per group. NC: Normal control, DC: Diabetic control. GL: Glibenclamide. Data analyzed by ANOVA followed by Dunnett's multiple test for comparison. ap< 0.01: Significant difference of diabetic control from normal control. **p<0.01, *p<0.05: Significant difference of treated groups from diabetic control on corresponding days

Diabetic control group showed significant decrease in liver SOD and liver CAT enzymes as compared to normal control as shown in **Table 4**.

Administration of herbal mixture (100 and 200 mg/kg) for 28 days showed significant improvement in liver SOD and liver CAT.

As shown in **Table 5**, body weight of STZ diabetic rats was significantly decreased at the end of 28 days in comparison to normal rats.

Treatment of diabetic rats with herbal mixture (100 and 200 mg/kg) and glibenclamide (4 mg/kg) for 28 days significantly (p<0.01) improved the body weight. Diabetic control group showed significant increase in food and water intake with simultaneous increase in urine output as compared to normal control. Diabetic rats treated with the herbal mixture (100 and 200 mg/kg) showed a considerable reduction in water intake with a simultaneous decrease in urine output.

TABLE 4: EFFECT OF HERBAL MIXTURE ON LIVER ANTIOXIDANT ENZYMES IN STZ-INDUCED DIABETES IN RATS

Experimental Groups	SOD(U/min/mg protein)	CAT(Moles of H_2O_2 /min/mg protein)
Normal control (NC)	6.1±0.16	41.66±1.2
Diabetic control (DC)	1.85±0.08[a]	25.33±0.84[a]
DC+ Herbal mixture (100 mg/kg)	5.2±0.08[**]	31.5±0.96[**]
DC+ Herbal mixture (200 mg/kg)	5.6±0.11[**]	36.16±1.45[**]
DC + GL (4 mg/kg)	5.8±0.1[**]	36.5±1.63[**]

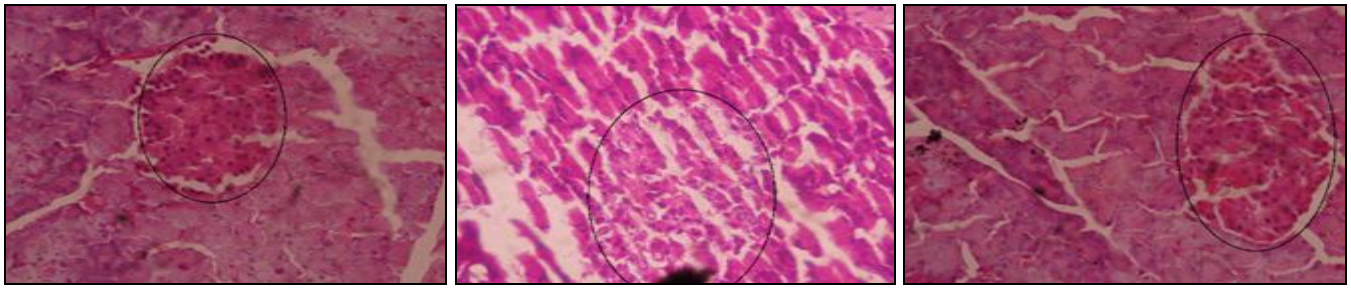
Values are expressed as mean ± S.E.M of six rats per group. NC: Normal control, DC: Diabetic control. GL: Glibenclamide, SOD: Superoxide dismutase, CAT: Catalase. Data analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple test for comparison. ap<0.01: Significant difference of diabetic control from normal control. **p<0.01, *p<0.05: Significant difference of treated groups from diabetic control.

TABLE 5: EFFECT OF HERBAL MIXTURE ON BODY WEIGHT, FOOD INTAKE, WATER INTAKE AND URINE OUTPUT IN STZ-INDUCED DIABETES IN RATS

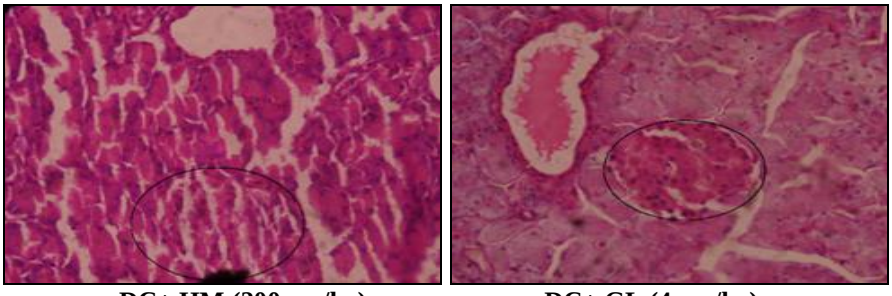
Experimental Groups	Body weight (g)	Food (g/day)	Water (ml/day)	Urine (ml/day)
Normal control (NC)	253±3.0	22.83±0.94	39±1.71	14±1
Diabetic control (DC)	168.83±2.89[a]	36.8±2.83[a]	65±2.82[a]	23±1.57[a]
DC+ Herbal mixture (100 mg/kg)	204.8±4.67[**]	30.16±1.6[*]	58±3.09[*]	20±1.02[*]
DC+ Herbal mixture (200 mg/kg)	214.33±3.19[**]	25.16±1.5[**]	52±1.44[*]	16±0.9[**]
DC + GL (4 mg/kg)	225.66±1.8[**]	20±1.43[**]	42.66±2.06[**]	15±1.23[*]

Values are expressed as mean ± S.E.M of six rats per group. NC: Normal control. DC: Diabetic control. GL: Glibenclamide. Data analyzed by ANOVA followed by Dunnett's multiple test for comparison. ap<0.01: Significant difference of diabetic control from normal control. **p<0.01, *p<0.05: Significant difference of treated groups from diabetic control

As shown in **Fig. 1**, STZ-treated pancreatic sections showed clear decrease in the area occupied by the beta cells probably due to reduction in the number of beta cells. Sections of pancreas treated with herbal mixture had clearly shown the protective effect on histology of pancreatic cells.



NC DC+HM (100 mg/kg)



DC+ HM (200 mg/kg) DC+ GL (4 mg/kg)

FIG. 1: EFFECT OF HERBAL MIXTURE ON HISTOPATHOLOGY OF PANCREAS IN STZ- DIABETIC RATS NC: Normal Control, DC: Diabetic Control, HM: Herbal Mixture, GL: Glibenclamide. The figure shows photomicrographs of histopathological sections of rat pancreas of different experimental groups. Photomicrographs are taken using an electronic bilobed microscope (Labomed, Mumbai) under 40X magnification.

DISCUSSION: The purpose of this work was to establish the anti-hyperglycemic effect of an herbal mixture of three plant extracts i.e., aqueous extract of *Alstonia scholaris* bark (AEAS), methanolic extract of *Aegle marmelose* leaves (MEAM), and aqueous extract of *Withania coagulans* fruits (AEWC)in streptozotocin-induced diabetes in rats and to focus on its possible mechanism of action.

A compound can produce anti-hyperglycemic effect by various means. It may show direct effect like insulin, it may promote glucose-dependent insulin secretion, it may improve insulin sensitivity, it may potentiate insulin effect, it may reduce glucose absorption from the intestine, it may stimulate the peripheral utilization of glucose by promoting the conversion of glucose into glycogen or it may stimulate the regeneration of islets of Langerhans in the pancreas of STZ diabetic rats [19]. For the present study, we have used the STZ-induced diabetes model in Wistar rats. It is now well established that STZ selectively destroys the pancreatic beta cells irreversibly, causing degranulation or reduction of insulin secretion and produces hyperglycemia [20-21]. It has been reported that in this model of diabetes mellitus, insulin is markedly depleted, but not absent [22-23]. The massive destruction of pancreatic beta cells after STZ injection is due to alkylation of DNA, thereby producing hyperglycemia. It accounts for drastic reduction in insulin level, which alters glucose utilization and metabolism.

Administration of herbal mixture for 28 days showed a significant anti-hyperglycemic effect in STZ-diabetic rats. Decrease in blood glucose started after one week of drug treatment and continued up to four weeks. To find out the possible mechanism by which herbal mixture understudy shows antidiabetic effect, we studied various biochemical parameters amongst which serum insulin is an important one. The ability of the herbal mixture to improve serum insulin levels in diabetic rats indicates its ability to stimulate beta cells and releasing insulin. Improvement in insulin level by herbal mixture shows that its anti-hyperglycemic activity may be due to insulin-like effect or it could be due to protection of the intact functional beta cells from further deterioration so that they remain active and continue to produce insulin.

Oral glucose tolerance test is a diagnostic test for diabetes mellitus. In non-insulin-dependent diabetes mellitus, the kinetics of insulin release in response to meal or glucose alters. Hence elevated postprandial blood glucose is observed. When a compound is screened for its antidiabetic activity, evaluation of its effect on the oral glucose tolerance test will give an idea of how the compound affects the parameters compared to a control group and inhibits hyperglycemia to a variable degree.

In the present study, oral administration of herbal mixture returned blood glucose level to almost fasting level within 60-90 min. It indicates ability of the extracts to improve glucose tolerance due to augmented glucose transport and increase in peripheral utilization of glucose.

Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals and consequently to the onset of oxidative stress which has been implicated in diabetes-associated complications [24]. Insulin deficiency in the diabetic state results in the impairment of glucose utilization leading to an increased generation of oxygen free radicals. Oxidative stress in diabetes has a significant effect in the glucose transport protein and in insulin receptor activity [25]. Scavengers of oxidative stress may therefore have an effect in reducing the increased serum glucose level in diabetes and may reduce its secondary complications [26].

STZ-induced hyperglycemia induces the generation of superoxide radicals and hydroxyl radicals which induces various injuries in surrounding organs and plays an important role in the partial or total alleviation of organ damage. Thus removal of superoxide and hydroxyl radicals is probably one of the effective defenses of a living body against diseases. Superoxide dismutase is an important defense enzyme that catalyzes the dismutation of superoxide radicals. Superoxide dismutase scavenges the superoxide ions produced as cellular byproducts and is a major defense for aerobic cells in combating the toxic effects of superoxide radicals [27]. Enzyme catalase is the hemoprotein that catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. Catalase reduces the hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisomes.

In the present study, a significant decrease in SOD and CAT activities is observed in the liver of diabetic rats when compared with normal control.

Improvement in liver SOD and CAT enzymes after treatment with herbal mixture indicates its beneficial action against the pathological alteration caused by the presence of superoxide and hydroxyl radicals. It also shows the ability of extracts to scavenge reactive oxygen species and thereby inhibit lipid peroxidation. The anti-hyperglycemic activity of herbal mixture is thus associated with decrease in glucose toxicity resulting in protection from the toxic effects of reactive oxygen species.

Increased non-enzymatic and auto-oxidative glycolsylation is one of the possible mechanisms linking hyperglycemia and the vascular complications of diabetes [28]. Hyperglycemia can enhance protein glycation and the erythrocytes are more prone to oxidative stress in diabetes. The longer the exposure of erythrocytes to hyperglycemia, the shorter is its life span. Glycosylated hemoglobin (HbA1c) is formed progressively and irreversibly over a period of time. The present study showed increased HbA1c levels in the diabetic control group. The herbal mixture treatment showed improvement in glycosylated hemoglobin as compared to diabetic control. Prolonged intake of the herbal mixture therefore may further reduce HbA1c levels and probably help in achieving better glycemic control.

The estimation of glucose content in rat hemidiaphragm is a commonly employed and reliable method for *in-vitro* study of peripheral uptake of glucose. Oral administration of herbal extract enhances the uptake of glucose by isolated rat-hemi diaphragm in diabetic rats. Thus anti-hyperglycemic action of herbal mixture could be due to its consequence of improved peripheral glucose consumption

Insulin deficiency and insulin resistance in type II diabetes decrease the peripheral uptake of glucose, increase hepatic gluconeogenesis and thereby decrease liver glycogen. Increase in liver glycogen content after administration of the herbal mixture and glibenclamide 29 in diabetic rats indicates its possible effect through increase in insulin secretion and thereby enhancing the activity of key enzymes of glycogen synthesis and by sensitizing the tissues like liver for uptake of glucose. Improvement in liver glycogen might also be due to the insulin-like activity of the herbal mixture. This indicates that one of the possible ways of antidiabetogenic action of this herbal mixture is by improvement of glycogenesis process in liver.

Insulin deficiency in diabetes decreases the peripheral uptake of glucose and synthesis of glycogen, thereby affecting the body weight of animals. Inadequate insulin levels facilitate the breakdown of muscle proteins to provide gluconeogenic precursors. Loss of body weight after administration of STZ may be due to the loss of degradation of structural proteins [30]. The failure of diabetic animals to gain weight during the course of time is also due to continuous excretion of glucose in urine [31]. Along with the decrease in body weight, diabetic rats showed weakness, polyuria, and polyphagia. Bodyweight improves after administration of herbal mixture in diabetic rats, which indicates its probable ability to improve insulin secretion, glycemic control, and also inhibition of gluconeogenesis.

CONCLUSION: The composite herbal mixture of extracts of *Aegle marmelose* leaves, *Alstonia scholaris* bark, and *Withania coagulans* fruits shows promising antidiabetic activity in STZ diabetic rats comparable to standard drug glibenclamide. The anti-hyperglycemic potential of the mixture is attributed to its pancreatic as well as extrapancreatic effects, and it could be because of the combined effect of active principal components like alkaloids, tannins, flavonoids, steroids, and saponins in the plants. The herbal mixture can be therefore pursued for its clinical usefulness in the management of diabetes mellitus and other associated complications.

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CONFLICTS OF INTEREST: All the authors declare that they have no conflict of interest.

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