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EVALUATION OF ANTI-DIABETIC ACTIVITY OF "ECO ENSULIN" IN EXPERIMENTALLY INDUCED DIABETES IN RATS

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ABSTRACT

Aim and Objective: This study is designed to perform preclinical studies of "Eco Ensulin" for regulatory purpose of Eco Remedies. Objective of the study is to screen the antidiabetic activity of "Eco Ensulin" in experimentally induced diabetes in rats.

Materials and Methods: 48 Albino Wistar Rats of either sex for study are used. The acute toxicity study of the Eco Ensulin was performed on female Albino Wistar Rats with body weight 200-250 as per OECD. After the acclimatization period, 6 rats were chosen as the normal control group. The rest of the 30 rats are divided into five groups (n=6) and are injected injection of streptozotocin (STZ) to produce hyperglycemia. The drugs are administered orally once daily for 45 days. Blood collected for various physical and biochemical parameters.

Results and discussion: The present study investigation showed that the metformin (50mg/kg) and Eco Ensulin (EE200, EE400, EE600) showed gain in the body weight as compared to DC. Also showed significant decrease in the food and water intake in metformin, EE600 and EE400, EE600 groups respectively, thus decrease in the polyphagia and polydipsia. It showed decrease in the serum insulin level in the treatment groups metformin (50mg/kg), EE400, EE600 groups as compared to DC. Also there was significant decrease in all the treatment groups metformin and Eco Ensulin (EE200, EE400, EE600) in HOMA-IR levels thus decrease in the insulin resistance and increase insulin sensitivity and helps in maintain the lipid parameters. Study also showed significant increase in the liver glycogen and muscle glycogen which indicates increase in glycogenesis and/or a decrease in glycogenolysis.

Conclusion: It can be concluded that the dose of EE 400 (400mg/kg) and EE600 (600mg/kg) are more potent than EE200 (200mg/kg) for all the parameters. Current finding suggests Eco Ensulin (Sugar Regulator) possess various pharmacological activities which may help in alleviating pathological abnormalities present in diabetes in rats further studies are suggested to explore the safety and efficacy of Eco Ensulin by suitable clinical trials.

Keywords: Eco Ensulin, Antidiabetic, Toxicity, Wistar, Streptozotocin, Pharmacological

INTRODUCTION

Diabetes mellitus has been defined by American Diabetes Association as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Indian physicians described also the disease and classified it as honey urine by the fact that ants were attacked by patient's urine **[1]**.

The term "diabetes" or "to pass through" was first used in 250 BC by the Greek Apollonius of Memphis.

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 and modified in 1985. An expert committee proposed two major classes of diabetes mellitus and named them insulin dependent diabetes mellitus (IDDM) or type I and non-insulin dependent diabetes mellitus (NIDDM) or type II **[2]**.

Type 1 diabetes (cell destruction, usually leading to absolute insulin deficiency) This type can be further classified as immune-mediated or idiopathic. The majority of type 1diabetes is of the immune-mediated nature, in which a T- cell mediated autoimmune attack leads to the loss of beta cells and thus insulin. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance). iii.

Type III or Gestational diabetes: Gestational diabetes mellitus (GDM) has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy **[3]**.

Secondary diabetes: Diabetes may develop as a consequence of other diseases or medication. Listed some causes of secondary diabetes, a term coined as "other types" of diabetes.

Epidemeology [4-6]

Global Prevelance of Diabetes

According to IDF number of people with diabetes worldwide and per region in 2017 and 2045 (20-79 years). Approximately 425 million adults (20-79 years) were living with diabetes. By 2045 this will rise to 629 million. The proportion of people with type 2 diabetes is increasing in most countries. 79% of adults with diabetes were living in low- and middle-income countries. The greatest number of people with diabetes were between 40 and 59 years of age. 1 in 2 (212 million) people with diabetes were undiagnosed. Diabetes caused 4 million deaths. Diabetes caused at least USD 727 billion dollars in health expenditure in 2017 – 12% of total spending on adults. More than 1,106,500 children were living with type 1 diabetes. More than 21 million live births (1 in 7 births) were affected by diabetes during pregnancy. 52 million people were at risk of developing type 2 diabetes.

Table1: Diabetes in India at a glance [7]

India At a Glance	2017	2045
Diabetes estimates (20-79 years)	Condense intervals in brackets	
Country prevalence, %	8.8 (6.7-10.9)	11.4 (8.8-14.0)
Age-adjusted comparative prevalence, %	10.4 (8.0-12.9)	10.4 (8.1-12.9)
Number of people with diabetes, in 1,000s	72,946.4 (55,473.0-90,198.1)	134,298.2 (103,390.8-165,171.9)
Number of people with undiagnosed diabetes, in1,000s	42,210.3 (32,099.4-52,193.0)	77711.4(59,826.9-95,576.5)
Proportion of undiagnosed cases, %	57.9	57.9
Number of deaths due to diabetes, in 1,000s	997.8 (763.2-1,198.3)	
Proportion of deaths due to diabetes in peopleunder 60 years, %	50.7	
Impaired glucose tolerance (20-79 years)		
Country prevalence, %	2.9 (2.2-5.8)	3.5 (2.6-6.7)
Age-adjusted comparative prevalence, %	3.3 (2.5-6.3)	3.3 (2.5-6.3)
Number of people with impaired glucose tolerance, in 1,000s	24,003.3 (18,327.5-48,398.9)	40,999.5 (31,091.4-78,622.8)
Healthcare expenditure due to diabetes (20-79 years)		
Total health expenditures, million USD*	8,713.0	13,340.8
Health expenditures per	119.4	99.3

Complication

Sustained high blood glucose levels (such as in the case of diabetes) is damaging to many organ systems. Therefore, T2DM is associated with severe long-term complications which contributes to high levels of morbidity and mortality **[8]**.

According to WHO 422 million people around the world have diabetes, particularly in low and middle income countries. For people living with diabetes, access to affordable treatment, is critical to their survival. Despite the use of advanced synthetic drugs for the treatment, use of herbal remedies is gaining higher importance because synthetic drugs have drawbacks and limitations like hypoglycemia, diarrhea, metallic after taste, nausea, headache, vomiting etc. The herbal drugs with antidiabetic activity are extensively formulated commercially because of easy availability and less side effects as compared to the synthetic antidiabetic drugs. So, present study is aimed to evaluate anti-diabetic effect of "Eco Ensulin" in experimentally induced diabetes in rats.

MATERIALS AND METHODS

Table 2: Poly herbal formulation

Contents	Each 100gm
Equal quantity of Curcumin (Turmeric)	10gm
Fenugreek seeds (Methi)	10gm
Bitter guard (Karela)	10gm
Syzygium cumini (Jamun)	10gm
Gymnena Sylvestre (Gudmari)	10gm
Trachyspermum Ammi (Ajwain)	10gm
Azadirachta Indica (Neem)	10gm
Black cumin (Kala jeera)	10gm
Phylanthus Emblica (Amla)	10gm
Natural excipients	10gm

Forty eight Albino Wistar Rats of either sex for study are used. Animals were maintained in polypropylene cages under environmentally controlled conditions with a temperature of (24±1°C), relative humidity of (60%±5%) and 12h/12h light dark cycle. The protocol of the experiment is approved by the Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) **[9-11]**.

Acute toxicity study [30]

The acute toxicity study of the Eco Ensulin was performed in Albino Wistar Rats on female with body weight 200-250 as per OECD guideline 423. Animal were fasted for 24hrs prior to beginning of this test. These were divided into two groups each group containing 6 animals each. One group was provided with water and another with EE at the dose of 2000mg/kg p.o. the animals were observed for 30 minutes and then periodically for first 24 hours special attention during the first 4 hours and thereafter daily for 14 days. The observations like sedation, convulsions, tremors, lethargy, death etc. were systematically recorded.

Phytochemical screening [12-15]

Following tests were performed according to standard procedures

- Test for carbohydrates: Molisch's test: Fehling's test: Benedict's test
- Test for Proteins and Amino acids: 1. Biuret's test: 2. Ninhydrin test:

- Test for Glycosides: 1. Legal's test: 2. Keller- Killiani test:
- Test for Alkaloids: Hager's test: Wagner's test:
- Test for Saponins: 1. Froth test:
- Test for Flavonoids: 1. Lead Acetate test: 2. Alkaline reagent test:
- Test for Triterpenoids and Steroids: 1. Salkowski's test: 2. Liebermann-Burchard's test:
- Test for Tannin and Phenolic compounds: 1. Ferric Chloride test: 2. Lead Acetate test:
- Test for Fats and Oils and Solubility test.

Dose calculation for rats [31]

•The doses of the formulation are calculated by extrapolating the therapeutic dose to rat dose on the basis of the body surface area ratio (conversion factor 0.018 for rats) by referring to the table of "Paget & Barnes"(Paget and Barnes 1964).

•Eco Ensulin HEALTHY SUGAR REGULATOR recommend the dosage of 1-2 teaspoon (1 teaspoon is equivalent to 4.2gm)

•Conversion of human dose to animal dose:

Human dose x 0.018 for rat weighing 200gm

i.e. 4200mg x 0.018 = 75.6mg/200gm/day approximately 76 mg/200gm rat

Conversion to dose/kg body wt.= 76mg x 5 = 380mg/kg rounded to 400mg/kg.

•Thus, the rat dose on the basis of surface area was found to be 400mg/kg and on the basis of the obtained rat dose, the test doses are on three levels low, therapeutic dose & high dose respectively.

In vivo model for Diabetes Type 2 [32-34]

Streptozotocin-nicotinamide induced rat model

Streptozotocin (STZ) is dissolved in 0.1 M citrate buffer (pH 4.5) and single intraperitoneal (i.p.) injection of 65 mg/kg is administered 15 min after the i.p. administration of 110 mg/kg of Nicotinamide (NIC) that is dissolved in normal saline.

Experimental design [16-20]

After the acclimatization period, 6 rats were chosen as the normal control (NC) group. They will received water and standard laboratory rodent diet throughout the experimental period. The rest of the 30 rats are divided into five groups (n=6) and are injected with a single intraperitoneal (i.p.) injection of streptozotocin (STZ) dissolved in 0.1M citrate buffer (pH4.5) 65mg/kg b.w., 15 min after the administration of 110mg/kg b.w. of nicotinamide dissolved in normal saline, and interval of 3 days to produce hyperglycemia. On the 4th day treatment is started. The drugs are administered orally using an intragastric tube once daily for 45 days continuously. Body weight of animals were measured throughout the experiment. After 45 day treatment is ended and the animals were fasted overnight and blood collected for various biochemical estimations. The animals were then sacrificed (under the influence of overdosed anaesthesia). The kidney, liver and pancreas were quickly excised, immediately rinsed in ice-cold saline; a portion of the organs were fixed in 10% neutral buffered formalin for histopathological study and the remaining portion were stored for further biochemical estimations.

Sample collection and parameters checked

Blood collection:

After 45 days, animals were fasted overnight and blood collected for various biochemical estimations. The animals were then sacrificed (under the influence of overdosed anaesthesia) and blood samples are collected by retro orbital puncture into plain sterile eppendorf tubes and allowed to clot at room temperature. Serum samples are separated by centrifugation at 3000rpm for 10 min and stored at -20°C until assay was performed **[21-23]**.

Evaluation parameter [24-30]

Body weight, food intake, water intake

Throughout the 45 days of study the body weight, food intake and water intake were monitored on a weekly basis. Food intake and water intake were measured as per cage basis.

Study of blood glucose level: Oral glucose tolerance test was performed at the end of the treatment.

Biochemical parameters

Estimation of Cholesterol, Estimation of Triglycerides (GPO Methods), Estimation of HDL, LDL, Liver Glycogen and Muscle Glycogen were studied.

Statistical Analysis

Data are expressed as mean ± standard error of mean (SEM). Statistical comparisons were made with one-way and two-way analysis of variance (ANOVA) appropriate using computer-based fitting program (Prism, Graphpad 8.0) Differences are considered to be statistically significant when $p < 0.05$.

RESULT AND DISCUSSION

Acute toxicity study of Eco Ensulin

Acute toxicity of Eco Ensulin was carried out on female albino rats. The animal received 2000mg/kg p.o. of Eco Ensulin. Study showed no mortality, till 14 days of the study period. This proves that the drug could be safely administered for acute treatments upto the dose of 2000mg/kg. From the obtained result of acute oral toxicity there was no significant change in various autonomic and behavioural response of rats and therefore it is considered to be safe.

Phytochemical screening of Eco Ensulin

It was found that carbohydrates, glycosides, alkaloids, saponins, flavonoids, triterpinoids, steroids, tannins, phenolic compounds, fats and oil and proteins and amino acids are present in the Eco Ensulin.

Effect of Eco Ensulin on body weight of diabetic rats

Table 3: Effect of Eco Ensulin on body weight of streptozotocin-nicotinamide induced diabetic rats

Groups n=6	Body weight(gm)							
	Day 0	Day 3	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
NC	221.66±9.45	221.66±8.33	230.83±6.37	238.33±4.01	240±3.65	236.66±6.14	239.16±5.23	238.16±7.03
DC	223.33±7.14	210.83±5.54#	187.66±2.90#	188.33±4.01#	191.66±4.01#	194.16±2.00#	205.83±2.00#	210±2.58#
SC	221.33±7.92	210±6.32	231.66±6.54*	236.66±8.81*	243.33±7.60*	244.16±5.54*	253.33±4.21*	253.33±4.21*
EE200	226.66±7.60	210±7.30	222.50±7.27*	228.33±6*	220±4.47*	226.66±4.94*	237.50±4.78*	245±5.62*
EE400	221.66±6	206.66±5.57	221.66±7.03*	231.66±5.42*	239.16±4.16*	246.66±5.10*	246.66±6.66*	245±6.11*
EE600	223.33±3.33	210±3.65	236.66±3.33*	244.16±20.71*	245±2.23*	248.33±4.77*	250.83±5.23*	249±5.50*

All values represents Mean ± SEM; n=6; #indicates significant difference from Normal control at P<0.05; *indicates significant difference from Disease control at P<0.05

Throughout the study, the diabetic animals showed significant (P<0.05) reduction in body weight when compared to the normal control animals. However, the Eco Ensulin and metformin inhibited the body weight reduction due to diabetes. This reduction in body weight is because of the insufficient insulin which prevents the body from getting glucose from the blood into the body cells to use as energy. Due to which the body starts burning fat and muscle for energy causing a reduction in overall body weight. Treatment with Eco Ensulin inhibited the b.w. reduction and this might be due to the active constituents that are present in Eco Ensulin and are reported for there increase in the insulin level and ß cell salvaging activity. Constituents like curcumin, polypeptide-P, mallic acid, vicine, gymnemic acid, quercetin, catechin which enhances the insulin level by decreasing the reactive oxygen species and salvaging the ß cells from the degeneration **[20-23, 29]**. This indicates that Eco Ensulin and metformin prevent the hyperglycemia induced muscle wastage.

Effect of various dose of Eco Ensulin on food and water intake of streptozotocin-nicotinamide induced diabetic rats

Average Food and water intake in animals of disease control group was significantly higher as compared to the normal control group. This increase in the food and water intake might be due to the polyphagia which is seen in the diabetic animals. There was reduction of the food and water intake in all treated group as compared to that of disease control but significant ($P<0.05$) change in the food and water intake is only observed in EE600 group and metformin treated as compared to disease control. It is confirms that Eco Ensulin can reduce polyphagia to a greater extent. The findings also suggest that the formulation does not alter normal metabolic parameters like food intake.

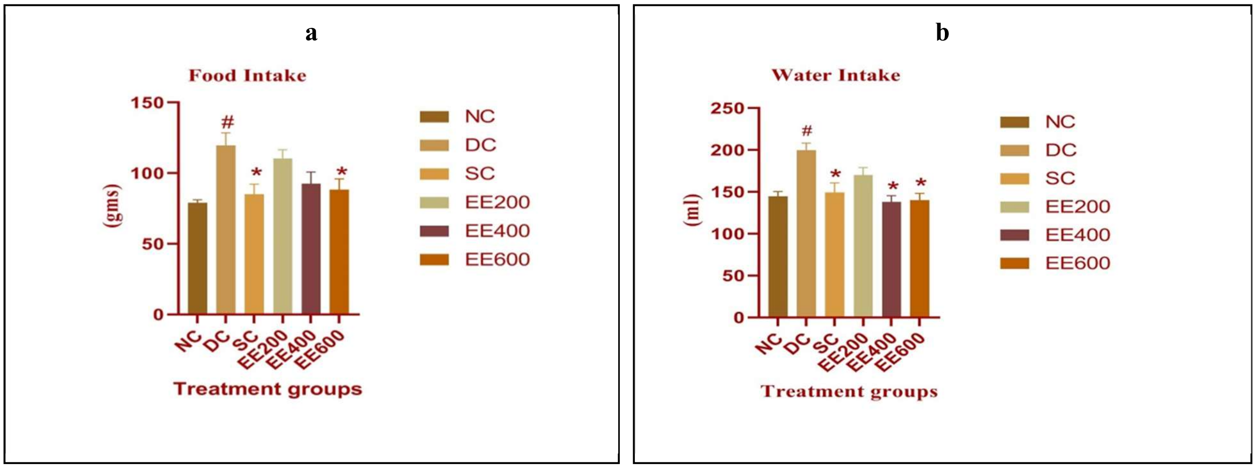

Figure 1: Effect of various dose of Eco Ensulin on a. food intake and b. water intake of streptozotocin-nicotinamide induced diabetic rats

Effect of Eco Ensulin on Oral Glucose Tolerence Test

Glucose level was found to be significantly increase in disease control group. Treatment with Eco Ensulin significantly improved glucose tolerance, as indicated by reduction in peak blood glucose levels at 1 and 2 h in treated groups during OGTT. Eco Ensulin might enhance glucose utilization by peripheral tissues and increasing the glycogen stores in the liver due to restoration of delayed insulin response because it significantly decreased the blood glucose levels in rats provided with oral glucose. This is because of the polyherbs that are present in the Eco Ensulin which contains several active constituents like flavanoids, terpenoids, polyphenolic acid, triterpenes which shows potent antidiabetic activity **[16-18]**. *M. charantia*, *P.Embillica* are the main components of the Eco Ensulin. It was reported that *M. charantia* improves glucose tolerance and suppresses postprandial hyperglycaemia in rats. It was reported that the clinical studies of *M. charantia* also showed the significant improvement in terms of OGTT due to the presence of the active constituents like polypeptide-p, vicinine, charantine **[22]**.

Table 4: Effect of Eco Ensulin on Oral Glucose Tolerence Test

Groups n=6	Blood glucose(mg/dl)					
	0min	15min	30min	60min	90min	120min
NC	105.16±2.12	142.83±4.04	166±4.76	148±4.20	129.66±6.15	107.5±2.51
DC	210.66±12.73#	261±15.22#	313.5±9.74#	348±15.51#	356.33±14.11#	310.66±7.57#
SC	113±7.37*	151.83±9.89*	183.5±8.36*	160.5±4.03*	131±4.04*	115.33±4.68*
EE200	138.16±8.17*	168.83±9.71*	211±15.65*	247.33±17.84*	217±14.43*	186.5±13.84*
EE400	116.66±4.16*	141.83±6.26*	179.5±5.48*	200.83±7.25*	159.83±5.22*	123.33±5.03*
EE600	107±3.97*	141.16±4.40*	179±4.92*	256.66±20.58*	195.83±12.85*	118.33±4.53*

All values represents Mean ± SEM; n=6; #indicates significant difference from Normal control at *P*<0.05; *indicates significant difference from Disease control at *P*<0.05

Effect of Eco Ensulin on Serum Insulin

Results shows effect of Eco Ensulin on insulin levels in rats. Hyperinsulinemia was observed in disease control group when compared with normal control group. Eco Ensulin treatment significantly (P<0.05) reduced the elevated levels of insulin when compared with disease control group. Metformin treatment also significantly (P<0.05) reduced the elevated levels of insulin when compared with Disease control group. The hyperinsulinemia in disease control groups could be either due to decreased hepatic clearance of insulin or by down-regulation of insulin receptors and desensitizing post receptor pathways, resulting in decreased insulin binding and degradation. However, Eco Ensulin treatment was found to be effective in reducing insulin levels of treatment groups thereby preventing hyperinsulinemia. It seems that Eco Ensulin exerts antihyperglycemic effect by attenuating hyperinsulinemia.

Insulin resistance (IR) is a characteristic pathophysiological feature of T2DM, and is defined as a state in which an abnormally higher amount of insulin is required to elicit a quantitatively normal biological response. In other words, IR refers to the inability of an insulin-sensitive cell to respond to normal physiological concentrations of insulin, which results in an impairment of the insulin induced glucose uptake and downstream metabolism by a cell. As tissues and organs become increasingly resistant to insulin, the pancreatic β-cells compensate by increasing insulin synthesis to maintain normal blood glucose levels. This leads to hyperinsulinemia high plasma insulin levels. Chronic overstimulation of the β-cells, which can be due to over nutrition and/or insulin resistance, leads to β-cell exhaustion and dysregulation and ultimately causes β-cell failure and insulin deficiency. IR and insulin deficiency contributes to hyperglycaemia which progresses to glucose intolerance and diabetes **[11]**.

Eco Ensulin contains Nigella Sativa which has thymoquinone. It was reported that thymoquinone has insulinotropic action where it can make partial regeneration to β cells of pancreatic islets, thus leading to improvement in their insulin production as well as its peripheral utilization. Thymoquinone has a down regulating effect on the gluconeogenic enzymes expression and the production of hepatic glucose besides its ability for diminishing intestinal absorption for glucose. Moreover, it can activate adenosine monophosphate-activated protein kinase (AMPK) in muscles and liver, thus inhibiting gluconeogenesis **[21]**.

Eco Ensulin also contains *M. charantia*. It was reported *M. charantia* extract can enhance insulin sensitivity and lipolysis **[22]**.

Eco Ensulin also contains *P. embellica* which has quercetin as an active constituent. It was reported that Quercetin improves fasting hyperglycemia by enhancing insulin sensitivity via α-glucosidase inhibition and enhanced insulin signaling in diabetic rats **[29]**.

The findings of previous study demonstrates the preventive effect of aqueous extract of fenugreek seeds on fat accumulation and dyslipidaemia due to inhibition of impaired lipid digestion and absorption, in addition to improvement in glucose and lipid metabolism, enhancement of insulin sensitivity, increased antioxidant defence, and downregulation of lipogenic enzymes. The insulinotropic activity of 4-hydroxyisoleucine and the compound being the active principle for the antidiabetic effect of fenugreek seeds have also been demonstrated by numerous other studies **[20]**.

Table 5: Effect of Eco Ensulin on Serum Insulin

Groups n=6	Serum Insulin µIU/ml
NC	8.89±0.56
DC	16.10±1.16#
SC	9.00±0.70*
EE200	12.30±1.03
EE400	9.00±0.88*
EE600	8.63±1.18*

All values represents Mean ± SEM; n=6; #indicates significant difference from Normal control at *P*<0.05; *indicates significant difference from Disease control at *P*<0.05

Effect of Eco Ensulin on Insulin Resistance

Results shows the levels of HOMA-IR, an index of insulin resistance. Disease control group showed significant (P<0.05) increase in HOMA-IR levels when compared with normal control group. Treatment with Eco Ensulin and Metformin significantly (P<0.05) prevents an increase in HOMA-IR levels as compared to disease control group. Study showed decrease in blood glucose levels, prevented hyperinsulinemia and improved glucose tolerance in treatment groups. These results suggest that Eco Ensulin can improve insulin sensitivity. HOMA-IR is a useful clinical index of hepatic insulin resistance. The results obtained clearly showed that Eco Ensulin treatment significantly ($P<0.05$) prevented the rise in HOMA-IR in Eco Ensulin and metformin treated rats.

Eco Ensulin contains *M. charantia*. Abundant biochemical data have shed light upon possible mechanisms of the anti-diabetic actions of *M. charantia* with the recurring theme being activation of the AMP-activated protein kinase system. Other studies suggested a role of the a- and g-peroxisome proliferator-activated receptors (PPARa and PPARg) which are pivotal in lipid and glucose haemostasis and may mitigate insulin resistance **[22]**.

Eco ensulin contains G. sylvestre which was reported for decreasing fasting blood glucose in streptozotocin diabetic rats that may be due to increase the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway or regenerate cells in pancreatic islets **[23]**.

Eco Ensulin contains *Syzygium cumini*. It was reported *Syzygium cumini* seed extract is composed of many phytochemicals, such as triterpenoids, anthocyanins, oleic acid, essential oils, glycosides, saponins and several members of flavonoids (e.g., rutin, quercetin, myricetin, myricitrin) which decreases the insulin resistance **[28]**.

Table 6: Effect of Eco Ensulin on Insulin Resistance

Group n=6	Fasting glucose (mg/dl)	Fasting insulin (µIU/ml)	HOMA-IR
NC	105.5±2.75	8.89±0.56	2.31±0.13
DC	250.83±11.69	16.10±1.16	10.08±1.04#
SC	106.16±2.67	9.00±0.70	2.35±0.17*
EE200	140.83±8.07	12.30±1.03	4.25±0.36*
EE400	115.66±4.65	9.00±0.88	2.61±0.35*
EE600	107.16±4.11	8.63±1.18	2.21±0.25*

Effect of Eco Ensulin on Serum Lipid levels

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. The excess of fatty acids in the plasma may support the hepatic conversion of fatty acids into phospholipids and cholesterol. These changes may usually lead to secondary complications of diabetes such as atherosclerosis and increased coronary heart disease **[9, 10]**. Eco Ensulin contains *M. charantia*. In the previous studies it was showed that *M. charantia* improves the serum and liver lipid profiles and serum glucose levels by modulating PPAR-γ gene expression **[22]**. Eco Ensulin contains G. sylvestre. It was reported that gymnemma leaf extract had favorable effects, on lipid metabolism of diabetic rats. Derangement of glucose, fat, and protein metabolism in diabetes results in the development of hyperlipidemia **[23]**.

As shown in table Diabetic control rats showed a significant increase in the levels of triglycerides, cholesterol, LDL and a decrease in HDL when compared with normal control group. The treatment of diabetic rats with Eco Ensulin and metformin showed decrease in the levels of triglycerides, cholesterol, LDL and increase in HDL when compared with diabetic control rats. Herein, the decreased level of Total Cholesterol, Serum Triglyceride, Serum LDL and the increased level of Serum HDL in Eco Ensulin treated groups confirm the positive role of Eco Ensulin in increased insulin action on cholesterol and fatty acid biosynthesis and intestinal cholesterol absorption.32 The results indicate lipid profiles viz cholesterol, triglyceride and LDL were normalized significantly (P<0.05) by Eco Ensulin administration. HDL plays a key role in protecting against heart disease because of its role in the transportation of excess cholesterol out of the body and is known as "good cholesterol". In the present study, Eco Ensulin significantly increased the HDL level in treated diabetic animals. The hypolipidemic action of the Eco Ensulin could be the result of retardation of carbohydrate and fat absorption due to the presence of bioactive components in fenugreek.

Table 7: Effect of Eco Ensulin on Serum Lipid levels

Groups n=6	Serum Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)
NC	74.97±2.19	93.99±4.20	43.52±1.25	35.47±4.17
DC	106.13±4.30#	123.21±3.04#	23.81±1.00#	78.17±1.77#
SC	65.49±6.19*	93.35±1.67*	48.89±1.11*	31.35±1.75*
EE200	76.21±1.47*	102.47±4.32*	30.29±2.38	56.94±5.24*
EE400	75.21±1.47*	92.88±3.66*	48.58±1.41*	29.26±4.97*
EE600	72.33±1.16*	86.24±4.19*	48.58±1.61*	23.23±4.15*

All values represents Mean ± SEM; n=6; #indicates significant difference from Normal control at *P*<0.05; *indicates significant difference from Disease control at *P*<0.05

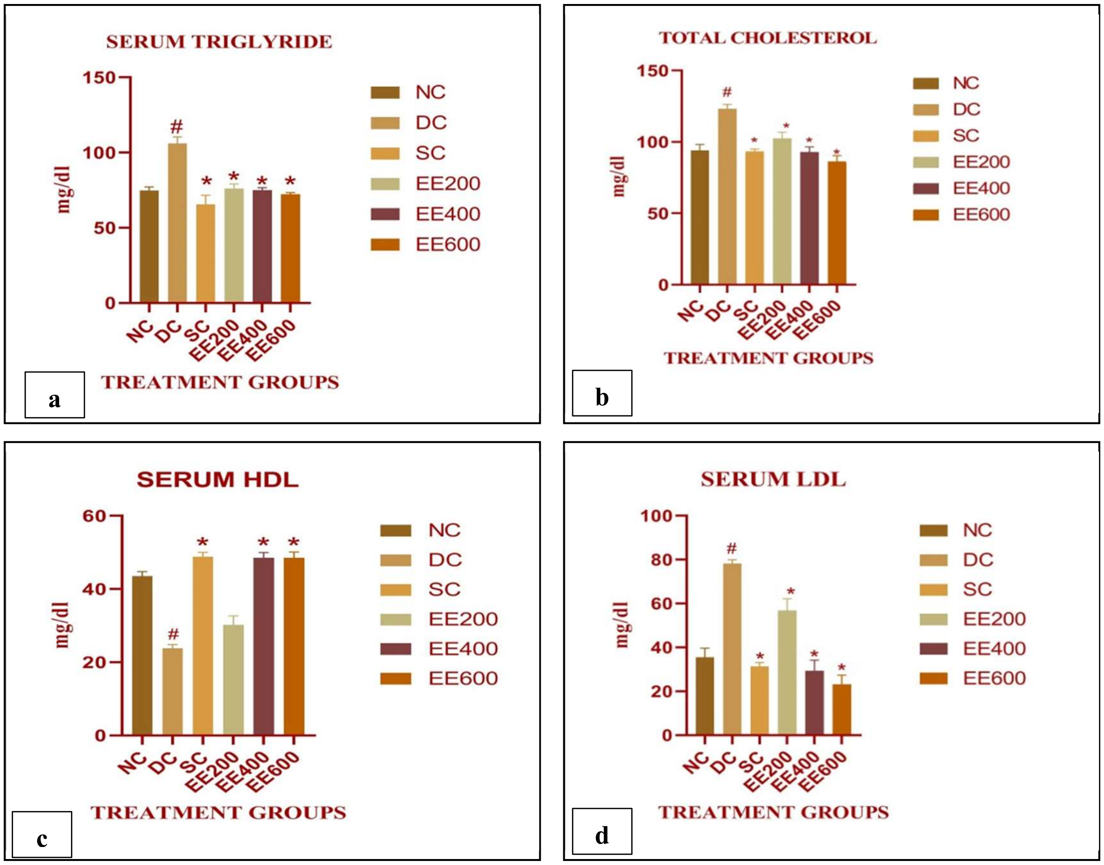

Figure 2: Effect of Eco Ensulin on (a) Serum Triglycerides (b) Total Cholesterol (c) Serum HDL and (d) Serum LDL

Effect of Eco Ensulin on Glycogen content

Eco Ensulin contain fenugreek which has galactomannan which stimulates glycogenesis and/or inhibits glycogenolysis in the liver of diabetic rats. The rise in the glycogen content of the liver after treatment can therefore be correlated with the decline in blood glucose levels **[20]**.

The glycogen content in the liver and muscle was significantly ($P < 0.05$) increased following treatment with Metformin and Eco Ensulin treated groups. An increase in the liver glycogen and muscle glycogen content after Eco Ensulin treatment might be brought about by an increase in glycogenesis and/or a decrease in glycogenolysis.

Table 8: Effect of Eco Ensulin on Glycogen content

Groups n=6	Liver glycogen (µg/mg)	Muscle glycogen (µg/mg)
NC	24.74±1.16	17.44±0.67
DC	15.46±0.84$^{\#}$	10.81±0.54$^{\#}$
SC	24.16±0.80*	16.71±1.08*
EE200	16.51±1.58	12.70±1.22
EE400	23.78±1.08*	16.32±0.92*
EE600	24.31±1.37*	17.38±0.86*

All values represents Mean ± SEM; n=6; #indicates significant difference from Normal control at P<0.05; *indicates significant difference from Disease control at P<0.05

CONCLUSION

Eco Ensulin (Sugar regulator) confirms the presence of carbohydrates, glycosides, alkaloids, saponins, flavonoids, triterpinoids, steroids, tannins, phenolic compounds, fats and oil and proteins and amino acids which are reported to posses potent antidiabetic activity. Here, the effect of Eco Ensulin was studied for beneficial antidiabetic effect in streptozotocin- nicotinamide induced diabetes in rats. The present study investigation showed that the metformin (50mg/kg) and Eco Ensulin (EE200, EE400, EE600) shows significant gain in the body weight ($p<0.05$). Also showed significant decrease in the food and water intake in metformin (50mg/kg), EE600 and EE400, EE600 ($p<0.05$) groups respectively, thus decrease in the polyphagia and polydipsia. Effective blood glucose lowering effect by OGTT was observed in all the treatment groups metformin (50mg/kg) and Eco Ensulin (EE200, EE400, EE600) ($p<0.05$).

Present study showed significant decrease in the serum insulin level in the treatment groups metformin(50mg/kg), EE400, EE600 groups. Also there was significant decrease in all the treatment groups metformin (50mg/kg) and Eco Ensulin (EE200, EE400, EE600) ($p<0.05$) in HOMA-IR levels thus decrease in the insulin resistance and increase insulin sensitivity and helps in maintain the lipid parameters are Total Cholesterol, Triglyceride, serum HDL, and serum LDL parameters. Study also shows significant increase in the liver glycogen and muscle glycogen which indicates increase in glycogenesis and/or a decrease in glycogenolysis.

Thus Current finding suggests Eco Ensulin (Sugar Regulator) possess various pharmacological activities which may help in alleviating pathological abnormalities present in diabetes in rats. Further studies are suggested to explore the safety and efficacy of Eco Ensulin by suitable clinical trials.

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None

CONFLICT OF INTEREST

None

REFERENCES

[1] Karamanou M. “Milestones in the history of diabetes mellitus: The main contributors.” *World Journal of Diabetes*, **2016**, *7*(1), 1.

[2] Baynest H. W., “Classification, Pathophysiology, Diagnosis and Management of Diabetes.” *Journal of Diabetes & Metabolism* **2015**, *6*(5), 1-9.

[3] https://www.who.int/news-room/fact-sheets/detail/diabetes

[4] https://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html

[5] Cho N. H., Shaw J. E., Karuranga S., Huang Y., da Rocha Fernandes J. D., Ohlrogge A. W., & Malanda B. "IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045." *Diabetes Research and Clinical Practice* **2018**, *138*, 271–281.

[6] Joshi S. R., "Diabetes Care in India." *Annals of Global Health* **2015**, *81*(6), 830–838.

[7] Sudagani J., & Hitman G. A., "Diabetes Mellitus: Etiology and Epidemiology". *Encyclopedia of Human Nutrition* **2012**, *2–4*, 40–46.

[8] Kahn S. E., Cooper M. E., & Prato S. D. "Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future." *The Lancet*, **2013,** *6736*(13), 1–16.

[9] Zaccardi F., Webb D. R., Yate, T., & Davies M. J. "Pathophysiology of type 1 and type 2 diabetes mellitus: A 90-year perspective" *Postgraduate Medical Journal*, **2016**, *92*(1084), 63–69.

[10] Hackett E., & Jacques N. "Type 2 diabetes pathophysiology and clinical features" *Clinical Pharmacist*, **2009**,*475*,475–478.

[11] Lankatillake C., Huynh T., & Dias D. A. "Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence - based medicinal plants" *Plant Methods*, 2019, 1–35.

[12] Tripathi K. "Essentials of Medical Pharmacology". 7th Edn. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi; 2013, 593-605.

[13] Vogel GH. And Vogel WH., "Drug Discovery and Evaluation" Berlin: Spinger Verla g; **1998**, pp947.

[14] King A. J. F., "The use of animal models in diabetes research." *British Journal of Pharmacology* **2012**, *166*(3), 877–894.

[15] Szkudelski T., "Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model." *Experimental Biology and Medicine* **2012**, *237*(5), 481–490.

[16] Arumugam G., Manjula, P., & Paari, N., "A review: Anti diabetic medicinal plants used for diabetes

mellitus." *Journal of Acute Disease* **2013**, *2*(3), 196–200.

[17] Patil R., Ahirwar B., & Ahirwar D., "Current status of Indian medicinal plants with antidiabetic potential: A review." *Asian Pacific Journal of Tropical Biomedicine* **2011**, 1(2), S291–S298

[18] Grover J. K., Yadav S., & Vats V. "Medicinal plants of India with anti-diabetic potential" *Journal of Ethnopharmacology*, **2002**, *81*(1), 81–100.

[19] Kokate C. K., Practical Pharmacognosy, 14th edn, vallabh prakashan, India, pp-110-114

[20] Anwar S., Desai S., Eidi M., & Eidi A., "Antidiabetic Activities of Fenugreek (*Trigonella foenum-graecum)* Seeds." *Nuts & Seeds in Health and Disease Prevention,* **2018,** 469-478

[21] Abdelrazek H. M. A., Kilany O. E., Muhammad M. A. A., Tag H. M., & Abdelazim A. M. "Black seed thymoquinone improved insulin secretion, hepatic glycogen storage, and oxidative stress in streptozotocin-induced diabetic male Wistar rats." *Oxidative Medicine and Cellular Longevity,* **2018**

[22] Joseph B., & Jini D., "Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency." *Asian Pacific Journal of Tropical Disease* **2013**, *3*(2), 93–102.

[23] Prabhu S., & Vijayakumar S. "Antidiabetic, hypolipidemic and histopathological analysis of Gymnema sylvestre (R. Br) leaves extract on streptozotocin induced diabetic rats." *Biomedicine and Preventive Nutrition* **2014**, *4*(3), 425–430.

[24] Pivari F., Mingione A., Brasacchio C., & Soldati L., "Curcumin and Type 2 Diabetes Mellitus: Prevention and Treatment." *Nutrients* **2019**, 11(8), 1837.

[25] Sanni O., Erukainure O. L., Chukwuma C. I., & Koorbanally N. A., "Azadirachta indica inhibits key enzyme linked to type 2 diabetes in vitro, abates oxidative hepatic injury and enhances muscle glucose uptake ex vivo." *Biomedicine & Pharmacotherapy* **2019**, 734–743.

[26] Aneesa N.N., Anitha R., Varghese S. "Antidiabetic Activity of Ajwain Oil in Different in vitro models". *Journal* *of pharmacy and bioallied science* **2019**, 142–147.

[27] Muruganathan U., Srinivasan S., & Indumathi D. “Antihyperglycemic effect of carvone: Effect on the levels of glycoprotein components in streptozotocin-induced diabetic rats.” *Journal of Acute Disease* **2013**, *2*(4), 310–315.

[28] Sharma S., Pathak S., Gupta G., Sharma S. K., Singh L., Sharma R. K., Dua K. “Pharmacological evaluation of aqueous extract of syzigium cumini for its antihyperglycemic and antidyslipidemic properties in diabetic rats fed a high cholesterol diet—Role of PPARγ and PPARα.” *Biomedicine and Pharmacotherapy* **2017**, *89*, 447–453.

[29] Srinivasan P., Vijayakumar S., Kothandaraman S., & Palani M. “Anti-diabetic activity of quercetin extracted from *Phyllanthus emblica* L. fruit: In silico and in vivo approaches.” *Journal of Pharmaceutical Analysis* **2018**, *8*(2), 109–118.

[30] OECD. Organisation For Economic Cooperation and Development. “Acute oral toxicity : acute toxic class method.” *OECD Guideline for Testing of Chemicals*, OECD423, **2001**,1–14.

[31] M.N. Ghosh, J.R. Vedasiromoni, “Fundamentals of Experimental Pharmacology”**,** 6th Edn, 173.

[32] Surana Y. S., Ashok P., & R. R. “Evaluation of Antidiabetic, Hypolipidemic and Antioxidant Activity of Polyherbal Formulation in Streptozotocin-Nicotinamide Induced Diabetes in Rats.” *International Journal of Pharmacy and Pharmaceutical Sciences* **2017**,*9*(10), 105.

[33] Shirwaikar A., Rajendran K., & Punitha I. S. R. “Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type 2 diabetic rats.” *Journal of Ethnopharmacology* **2005**, *97*, 369–374.

[34] Gogoi N., & Bhuyan B. “In Vivo Anti-Diabetic Activity Evaluation of the Bark of Cascabela Thevetia L. in Streptozotocin Induced Diabetic Rats” *International Journal of Pharmacy and Pharmaceutical Sciences*, **2017**, *9*(6), 48.

[35] Friedlandre Y, Kidorn M, Caslake M, Lamb T, McConnell M and Bar-On H (2000). "Low density lipoprotein particles size and risk factors of insulin resistance syndrome". Atherosclerosis"14,8, **2004**, 141-149

[36] Petchi R.R., Vijaya C., Parasuraman S. "Antidiabetic Activity of Polyherbal Formulation in Streptozotocin – Nicotinamide Induced Diabetic Wistar Rats" *Journal of Traditional and Complementary Medicine* Vo1. 4, No. 2, **2014,** pp. 108-117.

[37] Bharti S.K., Krishnan S., Kumar A. and Kumar A. "Antidiabetic phytoconstituents and their mode of action on metabolic pathways" *Therapeutic Advances in Endocrinology and Metabolism* Vol. 9(3), **2018,** 81–100.