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SCREENING OF ANTIDIABETIC ACTIVITY OF POLYHERBAL COMPOUND ON ALLOXAN INDUCED DIABETIC ANIMAL MODEL

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Abstract:

The ethanolic extract of Nigella sativa, Celastrus paniculatus and Cinnamomum tamala (ENCC) is used in this study to screen the anti diabetic activity in Alloxan induced diabetic rats. Diabetes was induced in rats using Alloxan 150 mg/kg i.p. The study was carried out for 21 days. The phytochemical screening showed the presence of flavonoids, steroids, terpenoids and phemolic compounds. The treatment with ENCC 250 mg/kg and 500 mg/kg effectively increased the body weight of diabetic rats when compared with disease control group. It was discovered that the blood glucose levels and HbA1c levels were reduced in ENCC 250 mg/kg and 500 mg/kg treated groups when compared with disease control group. It also reduced the biochemical parameters like Total cholesterol (TC), Triglycerides (TG), Low density lipoprotein-cholesterol (LDL) and increased the high density lipoprotein-cholesterol (HDL) when compared with disease control group. Histopathlogical studies of pancreas was performed In disease control group, showed apoptosis of beta cells were as treatment with ENCC 250 mg/kg and 500 mg/kg showed mild hypertrophy of islets of pancreas and normal beta cells. Thus, the result suggests that ENCC possesses antibiabetic activity.

Keywords: *HbA1c, Alloxan, Diabetes, Nigella sativa, Celastrus paniculatus, Cinnamomum tamala, retro orbital plexus.*

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INTRODUCTION:

In today's world we can see the major cause of ailment in humans is Diabetes Mellitus (DM). DM is considered as a severe complicated chronic condition that has given rise to high increase of illness on the planet. In DM there is disturbance in fat, protein and carbohydrate metabolism along with hyperglycemia which is studied as primary characteristics of the cause. The complete lack of hormone insulin is counted as the secondary characteristic cause [1].

Diabetes is divided in three types. They are categorized as Type 1 diabetes, Type 2 diabetes and gestational diabetes. Type 1 diabetes is also known as juvenile-onset diabetes is caused due to destruction of pancreatic beta cells causing insulin deficiency that shows symptoms such as polydypsia, polyphagia and polyuria [2]. Type 2 diabetes is also known as adult-onset diabetes is caused due to insulin resistance in liver and fat tissues leading to insulin deficiency [3].Gestational diabetes is the onset of glucose intolerance generally detected in second or third trimester during pregnancy [4].

In order to cure diabetes there is still no satisfactory drug available. But to reduce the side effects associated with allopathic treatment like insulin and oral hypoglycemic agents, patients are demanding to use natural products with antidiabetic activity. In recent times, the field of herbal drugs has seen a tremendous growth in its usage in many countries. The reason being is their natural origin and probably the less side effects caused by these herbal drugs. These drugs have been derived from medicinal plants, minerals and organic matter [5].

According to World Health Organization, there are more than 21,000 plants listed and out of which 2,500 species are found in India itself, among which 150 plant species are commercially used in India for the treatment of different diseases [6]. Hence in our research we planned to explore the antidiabetic activity of polyherbal compound consisting of *Nigella sativa, Celastrus paniculatus and Cinnamomum tamala.* Plants have been selected for the treatment of diabetes based on the presence of chemical constituents like alkaloids, flavonoids, terpenoids and glycosides. These constituents by different mechanism show their antidiabetic activity [7].

Nigella sativa is a flowering plant that belongs to Ranunculaceae family and is also used as a spice. It is commonly known as black cumin or kalonji. The presence of phytoconstituent thymoquinone is responsible for most of the pharmacological activities [8]. *Celastrus paniculatus*, a woody liana belongs to family of Celastraceae. It is commonly known as intellect tree or jyotishmati. It is used as anti inflammatory, analgesic, anti-oxidant and anti-bacterial agent [9]. *Cinnamomum tamala* belongs to family of Lauraceae. It is commonly known as tejpatta or bay leaf. It is used as antibacterial, nephroprotective, analgesic and anti-cancer agent[10].

MATERIAL AND METHODS:

Plant material

The seeds of *Nigella sativa, Celastrus paniculatus* and leaves of *Cinnamomum tamala* were collected and authentified from Dr. Shaik Mohammed Aliuddin Secretary: Hyderabad Unani Research Foundation. Hyderabad, Telangana State. The drug obtained was dried and then utilized for the study.

Preparation of extract

The seeds of *Nigella sativa* and *Celastrus paniculatus* were made into coarse powder in motar and pestle, leaves of *Cinnamomum tamala* were grinded to coarse powder in mechanical grinder. About 100 g of powder of the above mixture in the ratio of 1:1:1 is extracted with 500 ml of ethanol for 12 hour through Soxhlet extraction using Soxhlet apparatus. Coarsely grounded drug was taken in Soxhlet apparatus and ethanol (extracting solvent) is taken in round bottom flask and heated. The vapour gets condensed in condenser. The condensed solvent then drops into thimble containing the powder. When the chamber gets filled up and rises in siphon tube, the liquid content are sent into solvent present in round bottom flask. This is a continuous process and is carried on till no residue is sent from siphon tube. To avoid bumping during heating, the round bottom flask is filled with boiling chips. The product obtained is dried at room temperature.

Screening of Phytochemical Constituents

The preliminary phytochemical screening was carried out for ethanolic extract of *Nigella sativa, Celastrus paniculatus* and *Cinnamomum tamala* (ENCC) using standard procedures [11].

Animals

Wistar Albino rats of either sex were used for the study. The rats weighing between 150-200 g were procured from Sainath Animal Agency, Hyderabad. The temperature was maintained 22ºC (±3ºC) and relative humidity 50-60%.The animals were given pellet diet and drinking water *ad libitum*. The animal experimental protocol has been approved by our

Institutional Animal Ethical Committee with reference no: IAEC/SUCP/2019/07.

Induction of Diabetes

The Wistar Albino rats were fasted overnight before inducing diabetes. The freshly prepared Alloxan monohydrate (150 mg/kg) in normal saline was injected intraperitoneally (single dose). After one hour of administration of alloxan, rats were given 0.5% dextrose solution to overcome hypoglycemia for 24 hours. Later normal feed and water is given *ad libitum*. Three days after inducing diabetes, blood glucose level is checked and rats with glucose level more than 250 mg/dL were selected for the study.

Experimental Design

Wistar Albino rats of either sex were randomly divided into five groups each consisting of six animals as follows:

Group 1 (Normal control group) –This group was given 0.5% CMC p.o and normal pellet diet for a period of 21 days.

Group 2 (Disease control group**)** – This group was administered Alloxan 150 mg/kg BW i.p for inducing diabetes.

Group 3 (Standard) – The diabetic induced rats were treated with Glibenclamide 10 mg/kg BW in 0.5% CMC p.o for a period of 21 days.

Group 4 (ENCC 250 mg/kg) – The diabetic induced rats were treated with ENCC 250 mg/kg BW p.o in 0.5% CMC for a period of 21 days.

Group 5 (ENCC 500 mg/kg) – The diabetic induced rats were treated with ENCC 500 mg/kg BW p.o in 0.5% CMC for a period of 21 days.

Body weight

The body weight of the rats was recorded on 0^{th} day, 7^{th} day, 14^{th} day and 21^{st} day.

Collection of blood samples and biochemical parameter estimation

The blood samples were collected from retro orbital plexus of rats under mild ether anaesthesia on day 0, day 7, day 14 and day 21. These samples were centrifuged at 4000 RPM for 10 min and serum was isolated. This serum was subjected to blood glucose, glycosylated hemoglobin and lipid profile [Total Cholesterol (TC), Triglycerides (TG), Low density lipoprotein-cholesterol (LDL) and High density lipoprotein-cholesterol (HDL)] estimation. TC, TG and HDL were estimated using standard kits. LDL was calculated using Friedewald equation [12].

Histopathological study

On 21^{st} day one hour after drug administration animals were sacrificed by cervical dislocation. Pancreas was excised and kept in 10% formalin solution and subjected for histopathological studies.

Statistical Analysis

The statistical analysis was carried out using Graph pad prism 8.2.0. All results were expressed as Mean ± SEM. Groups of data were compared with analysis of variance (ANOVA) followed by Dunnett's multiple comparison test to identify significance ($p<0.001$, $p<0.01$, $p<0.05$) among groups.

RESULTS:

Screening of Phytochemical Constituents

The qualitative analysis of the phytohcemical constituents of ENCC showed the presence of carbohydrates, saponins, terpenoids, steroids, flavanoids, alkaloids, tannins, phenolic compounds and proteins.

Body weight

There was increase in body weight of ENCC 250 mg/kg and 500 mg/kg treated groups (145.12 g) and (150.51 g) when compared with disease control group (80.18 g). The Glibenclamide 5 mg/kg treated group also showed increase in body weight (160.29 g). The normal control group showed no significant change in body weight. These details were shown in Figure 1.

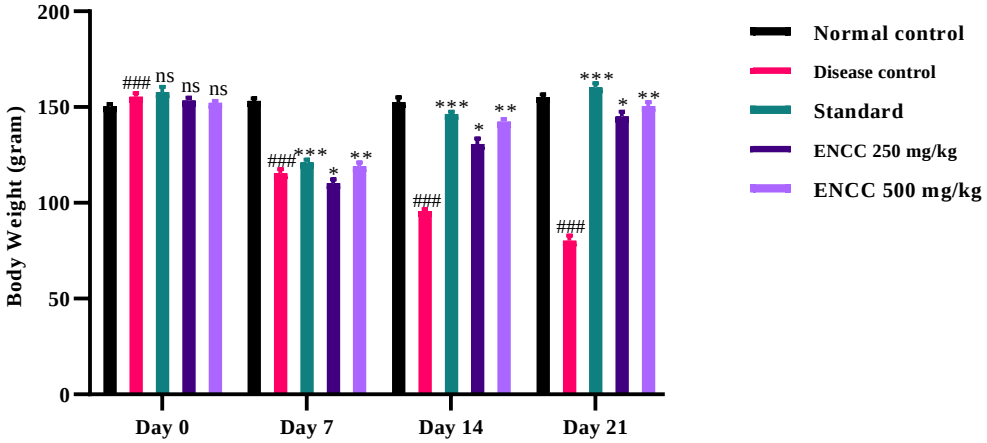

Figure 1: Effect of ENCC on body weight of experimental rats. The values are expressed as Mean ± SEM (n = 6).

One way ANOVA was carried out followed by Dunnett's multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Blood Glucose

There was decrease in blood glucose level of ENCC 250 mg/kg and 500 mg/kg treated group (157.16 mg/dL and 135.16 mg/dL) when compared with disease control group (358.92 mg/dL). Glibenclamide 5 mg/kg treated group showed decrease in blood glucose level (115.41 mg/dL). There was no significant change observed in blood glucose level of normal control group. These details were shown in Figure 2.

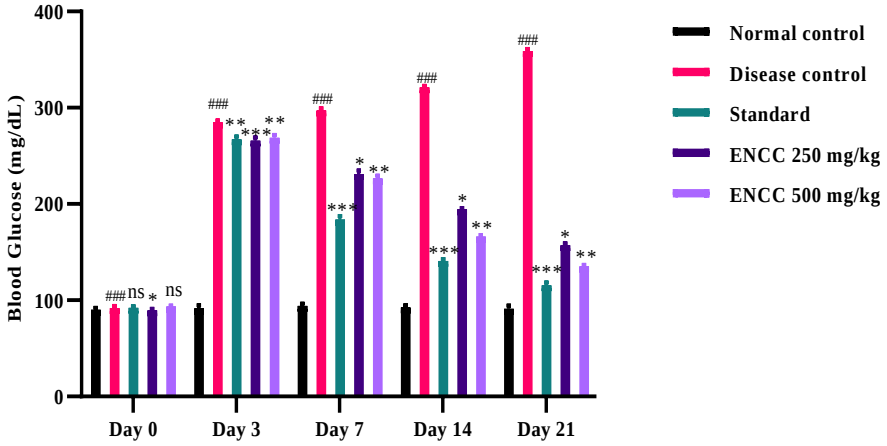

Figure 2: Effect of ENCC on blood glucose of experimental rats. The values are expressed as Mean ± SEM (n = 6).

One way ANOVA was carried out followed by Dunnett's multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Glycosylated Hemoglobin (HbA1c)

The HbA1c levels were found to be decreased in ENCC 250 mg/kg and 500 mg/kg treated group (5.92 % and 5.79 %) when compared with disease control group (6.60 %). In ENCC treated groups, levels were found to be similar with Glibenclamide 5 mg/kg treated group (5.47 %). These details were shown in Figure 3.

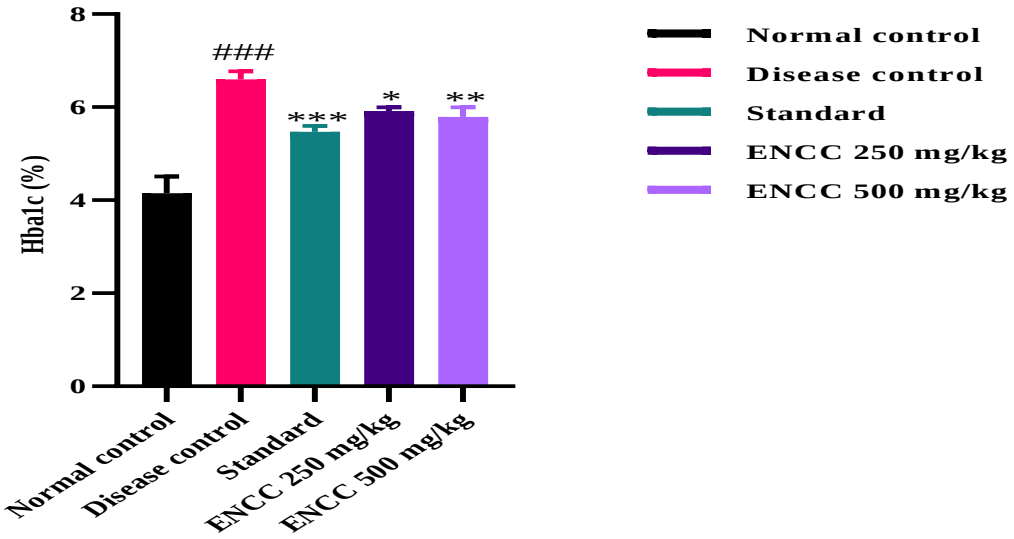

Figure 3: Effect of ENCC on HbA1c of experimental rats. The values are expressed as Mean ± SEM (n = 6).
One way ANOVA was carried out followed by Dunnett's multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group.

Lipid Profile

The TC levels were decreased in ENCC 250mg/kg and 500 mg/kg treated group (134.16 mg/dL and 116.16 mg/dL) when compared with disease control group (145.76 mg/dL). The TG levels were found to be decreased in ENCC 250 mg/kg and 500 mg/kg (92.34mg/dL and 85.42 mg/dL) when compared with disease control group (110.16 mg/dL). LDL levels were also found to be decreased in ENCC 250 mg/kg and 500 mg/kg (67.29 mg/dL and 51.19 mg/dL) when compared with disease control group (106.23 mg/dL). On the other hand the HDL levels were found to be increased in ENCC 250 mg/kg and 500 mg/kg treated group (26.16 mg/dL and 31.66 mg/dL) when compared with disease control group (20.66 mg/dL). These results were similar to the Glibenclamide 5 mg/kg treated group. These details were shown in Figure 4.

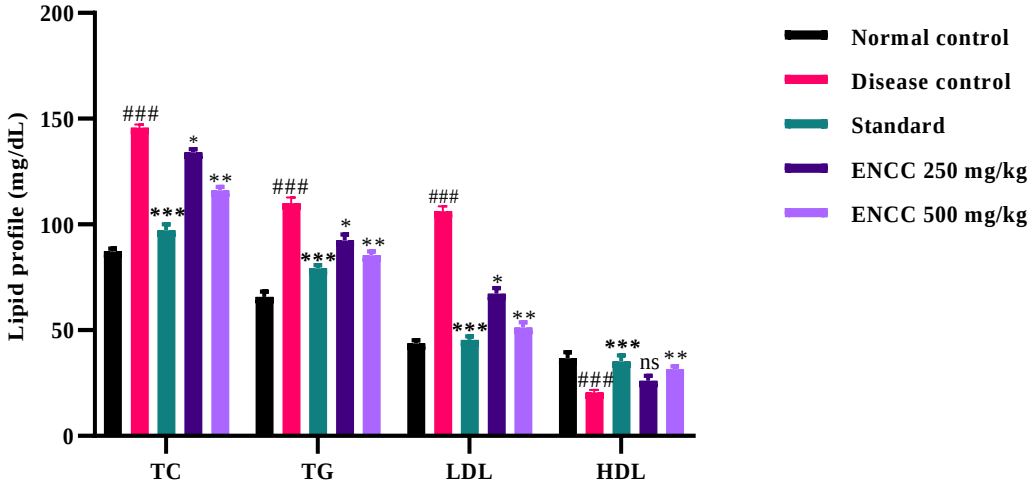

Figure 5: Effect of ENCC on Lipid profile of experimental rats. The values are expressed as Mean ± SEM (n = 6).
One way ANOVA was carried out followed by Dunnett's multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Histopathology Studies

The pancreas of rat belonging to control group showed normal beta cells in islets of pancreas (Figure: 6A). The disease control group showed apoptosis to beta cells with breakdown of islets of pancreas (Figure: 6B). The Glibenclamide treated group showed proliferation of beta cells with hypertrophy of islets of pancreas (Figure: 6C). ENCC treated group showed hypertrophy of islets of pancreas with proliferation of beta cell (250 mg/kg) (Figure: 6D) and normal islets with beta cells (500 mg/kg) (Figure: 6E).

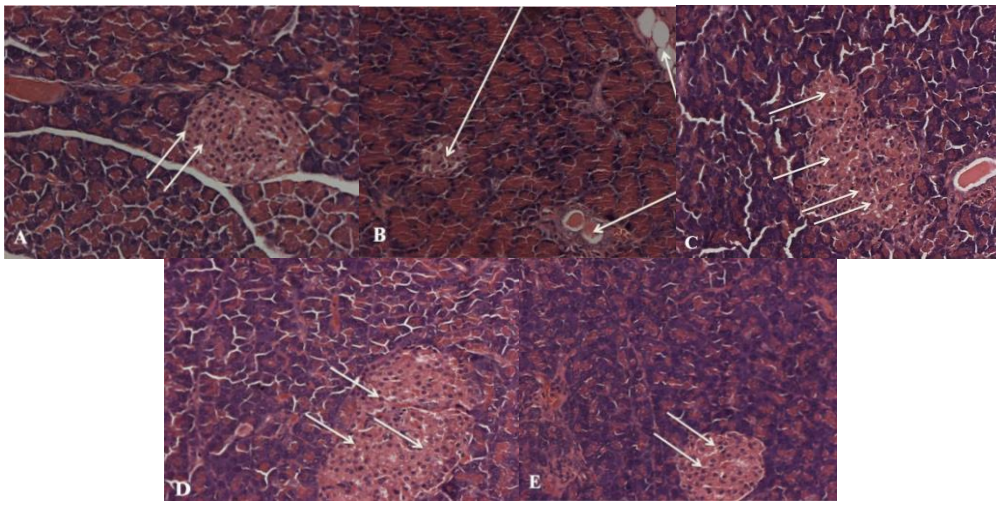

Figure 6: Histopathology of pancreas of A: Normal control B: Disease control C: Standard group D: ENCC 250 mg/kg E: ENCC 500 mg/kg

DISCUSSION:

Due to population explosion, it can be seen the risk of diabetes in this era is developing at faster pace. Despite of using the anti-diabetic drugs from the pharmaceutical industry, the herbal drugs treatment for diabetes can be considered more effective. The herbal medicines that comprises of different plant components cause less toxicity and show no side effects which are notable for the treatment of diabetes mellitus in the world.

In this study, a polyherbal formulation of *Nigella sativa*, *Celastrus paniculatus* and *Cinnamomum tamala* is used to treat Alloxan induced diabetes rats. The preliminary photochemical screening showed the presence of carbohydrates, saponins, terpenoids, steroids, flavanoids, alkaloids, tannins, phenolic compounds and proteins.

DM was induced in rats by administering Alloxan 150 mg/kg due to which sudden weight loss is observed in all the groups. In the present study, Alloxan induced disease control group showed decrease in body weight 80.18 g. On treatment with ENCC 250 mg/kg and 500 mg/kg there has been increase in body weight 145.12 g and 150.51 g respectively. These results were in accordance with the study conducted by Petchi RR et.al [13].

There was significant increase in blood glucose level in Alloxan induced disease control group 358.92 mg/dL which on treatment with ENCC 250 mg/kg and 500 mg/kg, showed decrease in blood glucose level 157.16 mg/dL and 135.16 mg/dL respectively. These results were in accordance with the study conducted by Sharma US and Kumar A [14].

Among the two doses of ENCC, ENCC 500 mg/kg showed significant anti diabetic activity which was evident from the results. As the polyherbal formulation contains *Nigella sativa*, which caused reduction in glucose level by the activation of enzyme AMPK to enhance glucose metabolism and also by inhibiting glucogenesis in liver [15].

Glycosylated hemoglobin (HbA1c) was one of the vital parameter in diagnosis of diabetes and also risk of developing other diabetic related complications. In this study, there has been increase in HbA1c value 6.60 % in Alloxan induced diabetic group. On treatment with ENCC 250 mg/kg and 500 mg/kg the results were 5.92 % and 5.79 % respectively. These results were in accordance with the study conducted by Rahimi P et.al [16].

One of the complications of diabetes is hyperlipidemia which leads to major cardiovascular risk factor. The rise in blood glucose was

accompanied with increased total cholesterol, triglycerides and LDL-cholesterol and reduction in HDL- cholesterol in diabetic rats. The impairment of insulin secretion results in enhanced lipid metabolism from adipose tissue to the plasma. It has been established that insulin deficiency leads to disturbance in metabolic processes which results in accumulation of lipids like cholesterol and triglycerides in diabetic patients. These results were in accordance with the study conducted by Sophia D and Manoharan S [17].

Histopathlogical studies were performed were disease control group showed apoptosis of beta cells were as treatment with ENCC 250 mg/kg and 500 mg/kg showed hypertrophy of islets of pancreas and normal beta cells [18].

CONCLUSION:

The administration of ENCC formulation showed effective increase in body weight when compared with disease group. The blood glucose levels and HbA1c value was found to be lowered in ENCC treated group when compared with disease control group. TC, TG and LDL levels are decreased in ENCC treated group when compared with disease control group were as HDL levels were increased.

Histopathlogical studies was performed, were disease control group showed apoptosis of beta cells were as treatment with ENCC 250 mg/kg and 500 mg/kg showed hypertrophy of islets of pancreas and normal beta cells. The preliminary phytochemical analysis of ENCC formulation showed the presence of flavonoids and phenolic compound which contribute to its anti diabetic activity.

The current study shows that the ethanolic extract of *Nigella sativa*, *Celastrus paniculatus* and *Cinnamomum tamala* showed the anti diabetic effect. However, it is also suggested for further investigation to study the absolute mechanism of the action of these plants at molecular levels that can be employed for anti diabetic treatment.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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