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Research Article

Anti-diabetic activity of polyherbal therapy in Streptozotocin induced diabetes mellitus

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ABSTRACT

Trigonella foenum, *Trachyspermum copticum* and *Nigella sativa* have been traditionally used for anti-diabetic activity and reported as having excellent anti-oxidant and anti-hyperlipidemic activity. The combination of extracts of these herbs has been potential of being developed as a therapy for the treatment of diabetes mellitus along with secondary complications due to its antioxidant and hypolipidemic activity. The objective was to evaluate antidiabetic activity of combination of methanolic extracts of *T. foenum, T. copticum, & N. sativa* in 1:1:1 proportion and elucidate its possible mechanism of action in Streptozotocin (STZ) induced diabetes model using Sprague Dawley rats. Methanolic extracts of three herbs at two dose levels (100 and 200 mg/kg), were administered orally to both male and female rats. The parameters studied included effect on the normal blood glucose level (normoglycemic model), oral glucose tolerance test (OGTT), fasting blood glucose (FBG), serum insulin, serum lipid profile, atherogenic index, and extrapancreatic effects like glucose uptake, liver glycogenolysis and liver glycogen content. Histopathological studies were carried out to determine the amount of pancreatic damage in each group. Normoglycemic and OGTT studies, demonstrated that both the doses showed significant hypoglycemic activity in treated rats. After 15 days of treatment with the combination of extracts, both the doses lowered the FBG by 64.1 mg % and 65.9 mg% respectively. A significant increase in serum insulin level was observed in the treated rats at both the doses. Elevated serum lipid levels were reversed to normal. The extract treatment also showed a significant increase in glucose uptake, elevated liver glycogen content and a significant decrease in glycogenolysis. The results demonstrated that the combination of extract possess significant antidiabetic activity in diabetic rats. Moreover, the combination reduced the hyperlipidemia; a complication of the metabolic disorder justifying its traditional claim and endorsing its potential as a promising therapy.

Keywords: diabetes; *Nigella sativa;* Streptozotocin HCl; *Trachyspermum copticum; Trigonella foenum*

INTRODUCTION

Diabetes is characterized by chronic hyperglycemia of a defined degree with or without glucosuria, hyperlipidemia and a tendency to develop ketoacidosis (Pitre, 1998). Oxidative stress has been implicated as a primary factor in the progression of diabetes mellitus (Kumaran and Joel, 2007). Hyperglycemia increases the generation of free radicals by glucose auto-oxidation resulting into damage to vital organs (Kim *et al.,* 2006). This results into secondary complications such as myocardial infarction, stroke, retinopathy, neuropathy and nephropathy leading to morbidity and mortality. This necessitates the need of a therapy with multifaceted mechanisms of lowering blood glucose level and correcting compromised physiological oxidative mechanisms in diabetic patients.

Currently available hypoglycemic agents have a number of limitations, such as the restricted spectrum of action, numerous adverse effects and high rates of secondary failure (Inzucchi, 2002) necessitating the need to discover efficient and safe hypoglycemic agents (Ramachandran, 2001). In traditional medicine, numerous medicinal plants have been used to control diabetes but very few attempts have been made to use them in combination for their probable synergistic effect.

Polyherbal therapy is a combination of many plant constituents from different plants, which can enhance the therapeutic efficacy by acting through multiple mechanisms. It constitutes synergistic, agonistic pharmacological agents within themselves working simultaneously in a dynamic way to produce higher therapeutic efficacy with minimum side effects (Tiwari and Rao, 2002).

Based on the folklore use of combination of *Trigonella foenum* (Fabaceae), *Trachyspermum copticum* (Apiaceae) and *Nigella sativa* (Ranunculaceae) in treatment

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of diabetes mellitus and resulting hyperlipidemia, we evaluated the above combination for its anti-diabetic activity to endorse the traditional claims.

Trigonella foenum commonly known as *Methi* is found all over India. The seeds are oblong, flattened or irregularly rhomboidal and yellowish-brown in color. The plant is very well known for its antidiabetic and hypolipedemic activity for a long time in traditional medicine (Akram E *et al.,* 2007). *Trachyspermum copticum* is commonly known as Bishop's weed. It is said to be native of Egypt and is cultivated in Iraq, Iran, Afghanistan, Pakistan and India. It consists of dried yellowish-brown ripe fruits possessing agreeable aromatic odour. It has been reported to have good antioxidant activity (Vaidyaratnam P, 2003). Blackseed (Nigella sativa L.) is known for its hypotensive effect (El Tahir *et al.,* 1993; Zaoui *et al.,* 2000; Bouchra *et al;* 2009). In India, it is mainly distributed in tropical to subtropical regions. The drug consists of dried seeds, which are black flattened, oblong, angular, or funnel-shaped. The seeds have aromatic odor, bitter taste and are a very good source of antioxidants (Gupta *et al.,* 2005).

Based on these facts an attempt was made to evaluate the synergistic effect of combination of methanolic extracts of *T. copticum, T. foenum* and *N. sativa* in 1:1:1 proportion at two dose levels (100 and 200 mg/kg) for the management of diabetes using STZ induced diabetic rat model.

MATERIAL AND METHODS

Reagent and chemicals

Streptozotocin HCl was procured from Sigma Aldrich Pvt. Ltd. Insulin ELISA kit was purchased from Mercodia Developing diagnostic Pvt. Ltd. The standard drug Metformin HCl was obtained as a gift sample from Bajaj Health Care Ltd. Glucose kit was procured from Accurex Biomedical Pvt. Ltd. Other biological kits like triglycerides, cholesterol and HDL were purchased from Merck India Pvt. Ltd., Mumbai.

Animals

Albino male and female rats of Sprague Dawley strain, weighing 200-250 g, were purchased from Bharat Serum & vaccines Ltd., Mumbai, India. The animals were housed in polypropylene cages with a maximum of three animals of same sex per cage in the animal house at ICT premises. The animals were maintained at the controlled temperature of 23 ± 1^{o}C, humidity of 55 ± 5%, in a 14 h light/10 h dark cycle. The rats were fed with standard pellet diet (Amrut laboratory animal feed, Maharashtra, India) and water *ad libitum*. They were allowed to acclimatize for one week before experimentation in the departmental animal house. Experimental protocols were reviewed and approved by the Institutional animal ethics committee. (Approval no. UICT/PH/IEAC/0109/02)

Preparation of extract, reference drug and Streptozotocin

A combination of methanolic extracts of *T. copticum, T. foenum* and *N. sativa* in 1:1:1 proportion was prepared using distilled water as a vehicle. 100 mg/kg and 200 mg/kg of combination were taken as a low dose (combination low dose - CL) and high dose (combination high dose - CH) respectively. Metformin was used as a reference drug and was administered orally 500 mg/kg as aqueous solution. STZ was prepared in a concentration of 65 mg/kg in freshly prepared cold saline solution (pH 4.4).

Experimental Procedure

Pharmacological evaluation of the antidiabetic activity of combination of methanolic extracts of *T. copticum, T. foenum* and *N. sativa* in 1:1:1 proportion was carried out using STZ induced diabetes model in Sprague Dawley rats.

Normoglycemic test in normal healthy rats

The normal healthy rats were randomly divided into 4 groups containing 6 animals each. Group I served as control. Group II and III received CL and CH respectively. Group IV served as positive control, which received the standard drug Metformin (500 mg/kg). Animals received the respective doses once daily for 15 days. Blood glucose waw determined on 0, 5, 10 and 15th day during the treatment period (Silawat *et al;* Vithlani *et al.,* 2008).

Glucose tolerance test in normal healthy rats

The normal healthy rats were randomly divided into 4 groups containing 6 animals each. Group I received no treatment. Group II was administered distilled water per oral. The group III and IV were administered CL and CH. Group IV was administered with Metformin (500 mg/kg, p.o.). All groups received glucose solution (1 g/kg), 30 minutes after the above treatments. Blood glucose levels were determined at 30, 60, 90 and 120 min after glucose administration (Nafisa *et al.,* 2008; Zhang *et al.,* 2000).

STZ induced diabetic model

The study consisted of 5 groups containing 6 animals each. Except control all the animals in each group were diabetic rats. A single dose (65 mg/kg, i.p.) of STZ HCl (dissolved in normal cold saline, pH 4.4) was used for induction of diabetes mellitus in rats. The induction of diabetes mellitus was confirmed after 3rd day of STZ treatment by estimation of elevated fasting blood glucose level (FBGL). Only those rats with FBGL ≥ 300 mg% was included in the study (Day 0). These rats were further divided in various groups as follows: Group I consisted of normal healthy rats, which received no treatment and served as control while group II served as diabetic control, which received distilled water (vehicle for administration of extract). Group III and IV were treated with CL and CH. Group V served as positive

control, which received Metformin (500 mg/kg p.o). Treatment with drugs was started on 4th day after STZ treatment (i.e., Day 1) and was continued for 15 days. All the drugs were given orally as a single dose in the morning. Blood glucose level was measured on 0th, 5th, 10th and 15th day during the treatment period. The other parameters measured along with glucose were insulin, triglycerides, cholesterol, HDL, VLDL, LDL and Atherogenic index. Blood was collected by retro-orbital puncture under anesthesia just before drug administration. Blood glucose, insulin and lipid profile was estimated by enzymatic methods using reagent kits.

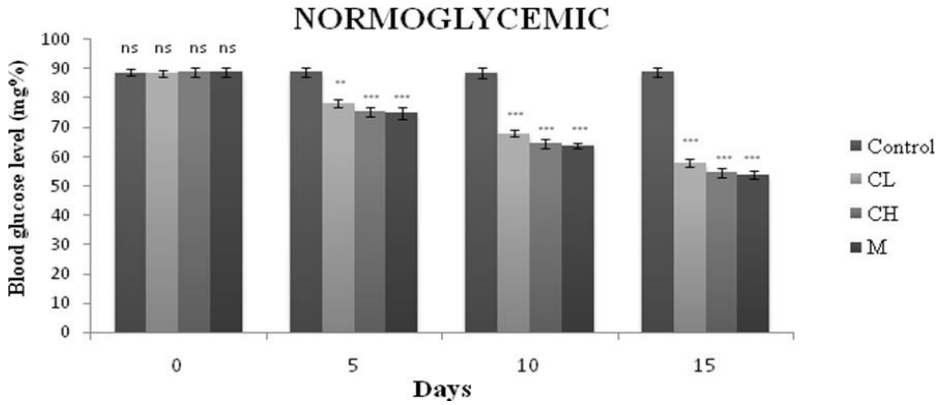

Figure 1: Effect of methanolic extract of CL and CH on normal blood glucose level of healthy rats

Treatment groups compared with control group.
**P<0.01 *P<0.05, ‡‡ P<0.01 & ‡ P<0.05.

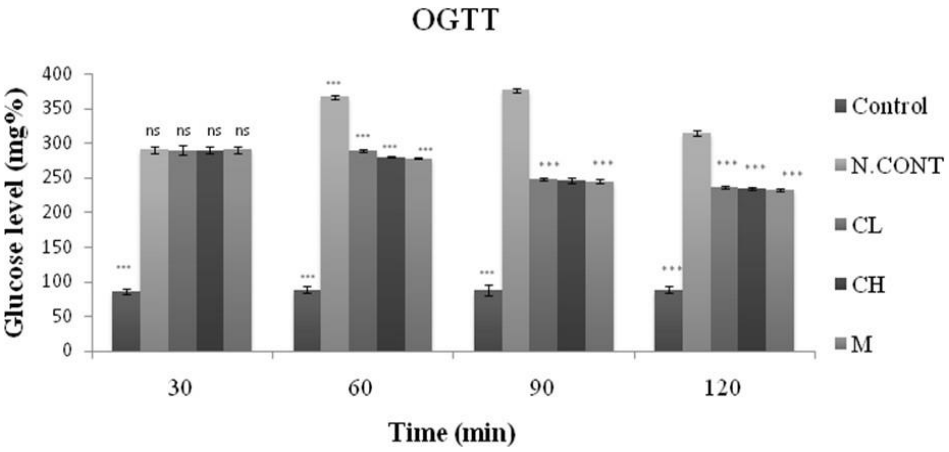

Figure 2: Effect of methanolic extract of CL and CH on glucose tolerance of glucose loaded rats

Negative control group compared with control group.
Treatment groups compared with Negative control group.
**P<0.01, *P<0.05, ‡‡ P<0.01 & ‡ P<0.05.

Effect on glucose uptake by hemidiaphragm and liver glycogenolysis

The rats treated daily for 15 days as described above were kept for overnight fasting and sacrificed on the next day under anesthesia. The hemidiaphragm and liver of the sacrificed rats were carefully excised and placed immediately in ice cold perfusate solution. The perfusion solution was prepared by adding the following constituents in distilled water: NaCl (0.687%), KCl (0.04%), $MgSO_4$ (0.014%), $CaCl_2$ (0.028%), $NaHPO_4$ (0.014%) and $NaHCO_3$ (0.21%). Another batch of similar perfusion solution was prepared to which glucose was added at a concentration of 400 mg%. This glucose perfusate solution was used to study glucose uptake and liver glycogenolysis. The hemidiaphragms were incubated in glucose perfusate solution at 37°C for 1.5 h with appropriate aeration to provide oxygen to the tissue and facilitate uniform mixing of the perfusate. At the end of an incubation period the perfusate solution was assayed to determine the glucose concentration. The hemidiaphragms were removed, rinsed in water and dried in an oven at 55–60°C for 4–5 h or until a constant, weight was obtained. The glucose uptake during the incubation period was calculated in terms of mg/100 mg dry weight of the diaphragm. Similarly, for determining liver glycogenolysis liver slices were incubated in the glucose enriched perfusate. The glucose concentration in the perfusate after the incubation period was determined in terms of mg/g of dry weight of liver (Nafisa *et al.,* 2008).

Determination of glycogen content in liver

The rats treated for 15 days were kept for overnight fasting on 15th day and were sacrificed on next day

under anesthesia. Liver was carefully excised, washed with saline and 1 gm of liver part was accurately weighed and stored at 0 °C until used. 1 gm of liver tissues was homogenized in 10 ml of hot ethanol for 2 min at tissue concentration of 100 mg/ml. The homogenized liver was centrifuged at a rate of 8000 X g for 20 minutes. 20 µl of the supernatant was used for initial glucose level estimation. The settled residue was collected and allowed to dry over water bath. 5 ml of KOH (10%) and 6 ml perchloric acid (52%) were added to this dry residue and left aside for 20 min at 0°C. The collected material was then centrifuged at 8000 X g for 15 min. 20 µl of the supernatant was removed for final estimation of glucose level. The glucose concentration was estimated by glucose estimation kit at 505 nm (Hassid W.Z, 1957). The glycogen content was calculated as:

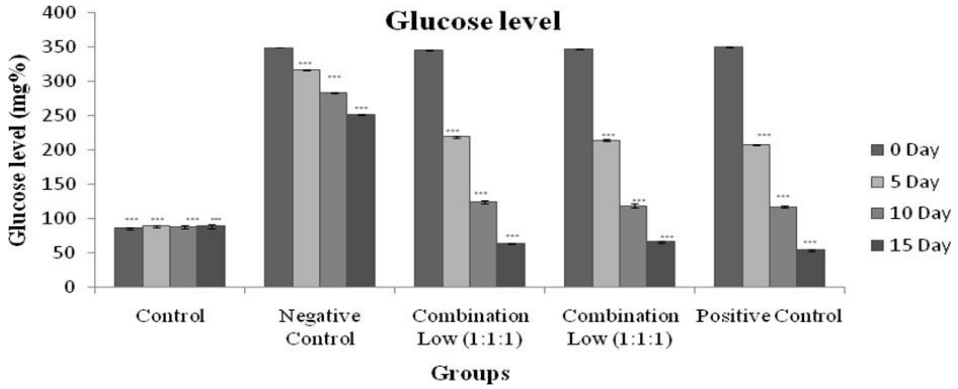

Figure 3: Effect of methanolic extract of CL and CH on blood glucose level of STZ induced diabetic rats

Negative control group compared with control group.
Treatment groups compared with Negative control group.
**P<0.01, *P<0.05, ‡‡ P<0.01 & ‡ P<0.05.

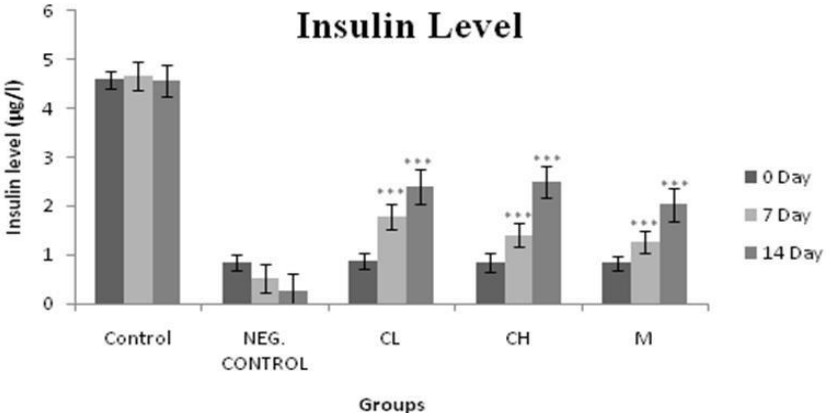

Figure 4: Effect of methanolic extract of CL and CH on blood insulin level of STZ induced diabetic rats

Negative control group compared with control group.
Treatment groups compared with Negative control group.
**P<0.01, *P<0.05, ‡‡ P<0.01 & ‡ P<0.05.

Histopathological study of pancreas of STZ diabetic rats

At the end of treatment, pancreas of control and all the treated groups were isolated and fixed in 10% (v/v) formalin. The tissues were processed and embedded in paraffin wax. Sections (5 µm) of these tissues were taken on glass slides and stained using a combination of hematoxylin–eosin and Gomori stain separately and were observed through the microscope for histopathological evaluations. Histopathological evaluation of the tissues was carried out at pathology laboratory, Bombay Veternary College, Mumbai, India. The histological parameters were transformed into numerical scoring system and finally expressed in terms of mean ± SEM.

Statistical analysis

The results were expressed as mean ± SEM. The data obtained was subjected to statistical analysis using one way ANOVA followed by Dunnett's test for comparison between negative control and test groups. A 'p' value < 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Normoglycemic test in normal healthy rats

It is generally recommended to start the evaluation of antidiabetic drugs with a simple test which should provide highly reproducible results and should not be time consuming. Normoglycemic and oral glucose tolerance test were such simpler and reproducible tests that

Table 1: Effect of methanolic extract of CL and CH on serum lipid profile

Oral treatment (n=6)	Triglycerides	Cholesterol	HDL	LDL	VLDL	Atherogenic index
	Mean± SEM					
Control	59.9±0.6	67.3±6.7	23.4±0.2	37.9±6.5	11.9±0.1	1.4±0.2
@N. control	138.1±3.0***	198.3±3.0***	13.7±0.3***	157.0±3.2***	27.3±0.6***	13.5±0.5***
CL	48.5±3.1***	67.9±2.3***	24.3±0.2***	33.9±2.1***	9.7±0.6***	1.7±0.8***
CH	37.6±3.1***	61.9±2.8***	24.2±0.3***	31.8±2.5***	7.5±0.6***	1.6±0.1***
@P. control	61.2±3.4***	96.8±3.2***	22.0±0.1***	62.5±3.3***	12.2±0.6***	3.3±0.1***

Negative control group compared with control group.
Treatment groups compared with Negative control group.
**P<0.01, *P<0.05, ‡‡ P<0.01 & ‡ P<0.05.
(@N. control – diabetic control and P. control – Metformin treated group)

Table 2: Effect of CL and CH on liver glycogen level, glucose uptake by hemidiaphragm and glycogenolysis by liver of STZ diabetic rats

Oral treatment (n=6)	Liver glycogen g/100g	Glucose uptake by hemidiaphragm mg/100mg	Glucose transfer by liver mg/g	Pancreas histoarchitecture necrotic changes & persistence of islets of Langerhans*
	mean± SEM			
Control	2.5±0.5	20.3±0.2	25.0±0.4	0.2±0.01
@ N. control	0.6±0.4***	10.4±0.3***	42.9±0.5***	2.8±0.02
CL	1.6±0.4***	36.1±0.4***	18.1±0.4***	2.8±0.02
CH	1.7±0.4***	37.4±0.4***	17.0±0.4***	2.8±0.01
@ P. control	1.5±0.6***	36.0±0.1***	19.2±0.5***	2.8±0.02

Negative control group compared with control group.
Treatment groups compared with Negative control group.
**P<0.01, *P<0.05, ‡‡ P<0.01 & ‡ P<0.05.
*Scoring system – 0 – Normal, 1 – mild, 2 – moderate, 3 – severe
(@ N. control – diabetic control and P. control – Metformin treated group)

were used to evaluate the effect of combination of methanolic extract of *T. copticum, T. foenum* and *N. sativa* in 1:1:1 proportion at two different doses (100 and 200 mg/kg) and Metformin (500 mg/kg) in normal and glucose loaded rats. Fig.1. depicts hypoglycemic effect of single oral administration of CL and CH at doses of 100 and 200 mg/kg respectively in normal healthy rats. Rats treated with CL (100 mg/kg) dose showed a significant fall of 34.68% and the one treated with CH (200 mg/kg) dose showed a significant fall of 38.46% in fasting blood glucose level on 15th day of treatment. Rats treated with Metformin (500 mg/kg) dose showed a significant fall of 39.39% in FBGL. Experimental finding of these studies suggested that CL and CH produced a significant blood glucose level lowering in normal rats and were found to be comparable to the standard drug Metformin. This indicated adequate care needed to be taken while administering this combination to normoglycemic population as it would produce hypoglycemia.

Glucose tolerance test in normal healthy rats

Fig.2. depicts hypoglycemic effect of single oral administration of CL and CH at doses 100 and 200 mg/kg in glucose loaded rats. The dose of 100 mg/kg of CL produced a maximum fall of 18.61% and dose of 200 mg/kg of CH exhibited 19.35% fall of BGL after 2 h of glucose administration. The standard drug Metformin produced a fall of 19.71% of BGL after 2 h of glucose administration. Thus, the fall produced by CL and CH was found to be comparable with standard drug Metformin. It is generally recommended to start with simple tests, which should provide highly reproducible result and should not be time consuming. The oral glucose tolerance test (OGTT) is a well-accepted and frequently used assay to screen preliminary hypoglycemic activity. Glucose levels are the starting point. The change in blood glucose can be periodically observed after a glucose load. The test gives an impression on the reactivity of the organism and the handling of the elevated glucose when a test compound is present. The lowering of the glucose can be better seen in this type of assay of glucose tolerance.

Among the three herbs used in combination therapy, *Trigonella foenum* is known for lowering blood glucose, blood lipid levels and protecting from hyperglycemia induced secondary complications. Thymol, present in fruits of *Trachyspermum copticum* is well established as an antioxidant, thus contributing to its protective action against hyperglycemica induced oxidative stress.

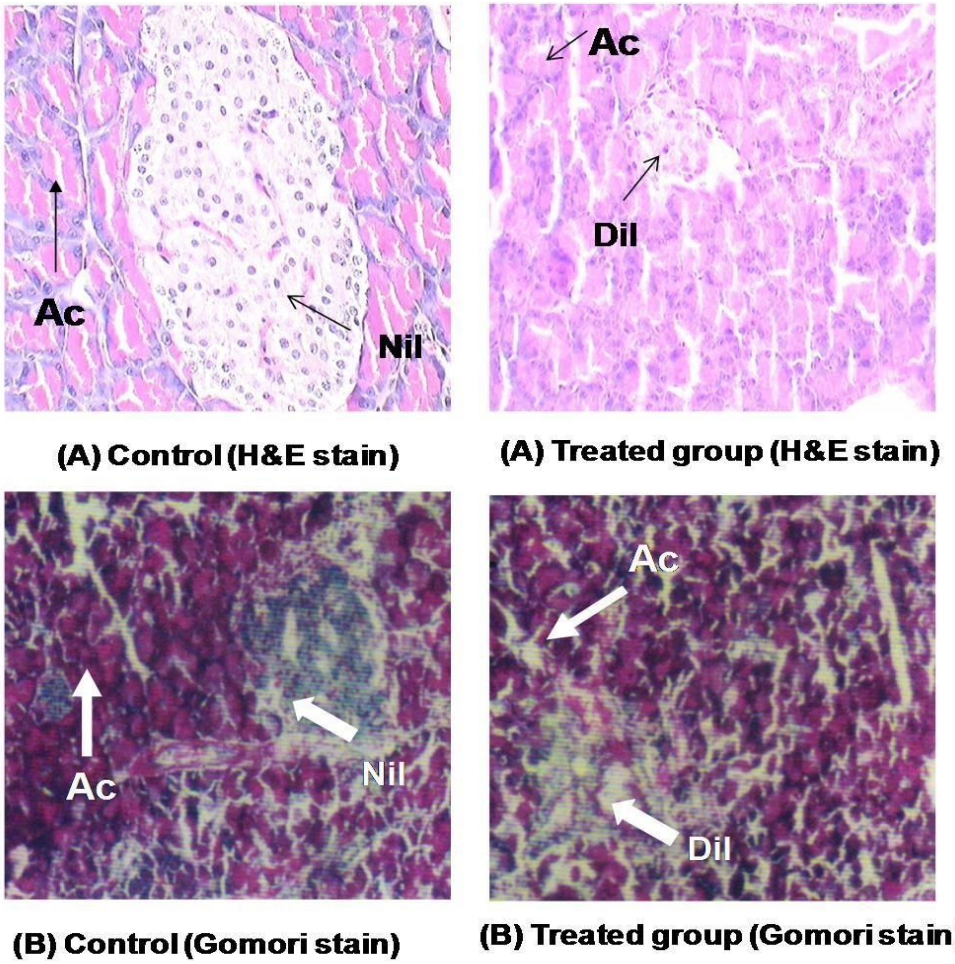

Figure 5: Representative photomicrographs of pancreas of control and treated groups

(A) Hematoxylin–eosin (B) Gomori stain, respectively [Ac – Intact acinus cells, Dil – degenerated islets of Langerhans, Nil – Normal islets of Langerhans. The organ sections were observed under microscope (40 ×).

Thymoquinone, the major constituent of seeds of *Nigella sativa* is reported to possess significant antidiabetic activity by enhancing the peripheral glucose uptake. Moreover, it is reported to improve beta cell function and state of insulin resistance in Type II diabetic patients. So the herbs in combination exhibited synergistic activity in surmounting diabetes mellitus and complications associated with the same.

STZ induced diabetic model

STZ was used to induce diabetes in Sprague Dawley rats. Diabetic rats showed the significant increase in blood glucose and lipid level and decreased insulin level in comparison to normal healthy rats in the initial stages.

Administration of CH, CL and Metformin restored the above parameters significantly towards normal. The effect of CL (100 mg/kg) and CH (200 mg/kg) was comparable with that of Metformin (500 mg/kg). CL and CH elicited a hypoglycemic effect in STZ induced diabetic rats as observed by the decrease in blood glucose levels determined on various days during the study. The initial blood glucose levels of the diabetic rats selected for the study were in the range of 340-360 mg%. In the diabetic rats treated with CL (100 mg/kg) and CH (200 mg/kg), the blood glucose level decreased steadily to 64.1 mg% and 65.9 mg% respectively, on the 15th day. Metformin (500 mg/kg) lowered the blood glucose level to 54.2 mg% following a 15 day treatment. The effect of CL and CH was comparable to that of marketed drug Metformin.

Insulin level of STZ induced untreated group (0.245 µIU/ml) was found to be significantly lower than that of a non diabetic control group (4.59 µIU/ml) indicating successful establishment of diabetes melitus with STZ. Groups treated with CL and CH have shown a significant elevation in insulin level of (2.39 µIU/ml) and (2.49 µIU/ml) as compared to standard drug Metformin with (2.03 µIU/ml). The increased serum insulin levels suggested that both; CL and CH have ability as an insulin secretogauge to enhance the secretion of insulin from the pancreatic beta cells of the islets of Langerhans.

Determination of lipid profile

Further to evaluate the effect of CL and CH on diabetes induced hyperlipedemia various biochemical parameters were evaluated following 15 days treatment in diabetic rats. All the groups except non diabetic control showed elevated serum triglycerides, cholesterol, LDL, VLDL and atherogenic index level. Untreated diabetic

rats exhibited significantly higher values of lipids profile compared to that of the control group. On the other hand, CL and CH treatment significantly lowered and restored the lipid values to normal comparable to that of positive control, Metformin. Moreover, CL and CH produced a significant restoration of decreased HDL level to normal. Thus, the combination could also prove beneficial in reducing diabetes induced complications, mainly due to resultant hyperlipedemia.

Determination of glucose uptake by hemidiaphragm

With a view to determine the extra pancreatic effects of CL and CH ex-vivo studies were planned to measure glucose uptake using hemidiaphragm. Glucose uptake in CL and CH treated animals was significantly higher as compared to untreated diabetic rats (CL: 36.1 ± 0.4 mg of glucose/100 mg hemidiaphragm, CH: 37.4 ± 0.4 mg of glucose/100 mg hemidiaphragm, untreated: 10.4 ± 0.3 mg of glucose/100 mg hemidiaphragm, respectively) (Table 2). Moreover, the glucose uptake in rats treated with CL and CH was found to be comparable (slightly higher although not significantly higher) to that of Metformin. In case of glucose uptake, a glucose molecule has to be phosphorylated to glucose-6-phosphate for intracellular utilization of the same in peripheral tissue. This process is enhanced by glucokinase enzyme. At the same time, glucose transporters like GLUT4 are responsible for carrying glucose molecule inside the cells. Insulin is known to play a vital role in up regulating glucokinase enzyme and GLUT4 transporters. However, in diabetic condition, both the enzyme and transporter are compromised leading to inhibition of glucose uptake. Ex- vivo studies reveled in that CL and CH have shown an enhanced glucose uptake (as seen by the stimulatory effect on glucose uptake in the rat diaphragm) which indicated that they probably have a direct up regulating effect on glucokinase enzyme and GLUT4 transporters, or it may have an indirect up regulating effect by enhancing insulin secretion and the subsequent transport of excess blood glucose from systemic circulation into the peripheral tissue.

Determination of liver glycogenolysis

In liver glycogenolysis studies, untreated diabetic rats showed very high glycogenolysis in liver. Rats treated with CL and CH could restore the glycogen content in the liver with significantly reduced glycogenolysis. The results of CL and CH were found to be comparable to that of marketed drug Metformin. Phosphorylase enzyme is responsible for the breakdown of glycogen into glucose molecule and thus enhancing glycogenolysis process. In healthy conditions, insulin is known to play an important role in inhibiting phosphorylase enzyme, but in diabetic condition either due to limited insulin secretion or resistance to its action, phosphoryalase enzyme is upregulated leading to enhanced glycogenolysis. Results suggested that CL and CH decreased glycogenolysis and enhanced glycogenesis in the liver of treated rats, which may be due to direct down regulating effect on phosphorylase enzyme or indirect down regulation of the same via enhancing insulin secretion.

Determination of glycogen content in liver

Results of liver glycogen content are illustrated in (Table 2). Untreated diabetic rats from the negative control group exhibited the least values of glycogen content 0.6 ± 0.4 g/100g of liver. In contrast, rats treated by CL and CH exhibited significantly higher glycogen content 1.6 ± 0.4 and 1.7 ± 0.4 g/100g of liver respectively compared to marketed drug Metformin 1.5 ± 0.6 g/100g. Results suggested that a significant restoration of glycogen was seen in case of treatment with CL and CH. These findings are in good agreement with the findings about the insulin level. Glycogen synthetase enzyme is responsible for synthesizing glycogen from glucose molecules in liver. Insulin facilitates glycogen synthesis by stimulating the enzyme glycogen synthetase. In diabetes, glycogen level is found to be decreased due to down regulation of glycogen synthetase enzyme. Our insulin level studies showed the increased levels of insulin in both CL and CH treated groups compared to that of Metformin. The increased glycogen content due to the extract treatment may either be due to direct up regulating effect on glycogen synthetase enzyme or indirect up regulation of the same by enhancing insulin secretion.

It is evident from the results that CL and CH treatment enhanced insulin secretion. Stimulatory effect of insulin on GLUT 4 transporters and up-regulation of glucokinase enzyme may be responsible for enhanced peripheral utilization of glucose seen after treatment with CL and CH. Enhanced glycogen content and a decreased glycogenolysis seen after treatment with CH and CL may be due to down regulation of phosporylase enzyme and/or up regulation of glycogen synthetase enzyme. This correction of impaired energy metabolism in DM needs to be explored further for understanding the correct mechanism of action of the combination.

Histopathological study of pancreas of STZ diabetic rats

Histopathological studies confirmed that there was no appreciable retention of histological architecture of the islets of Langerhans on STZ treatment or any type of regeneration of pancreatic islets of β cells with the drug treatments. All the groups indicated 60-70% of pancreatic β cell damage same as that seen in diabetic control. Normal healthy animals from the control group exhibited intact islets of Langerhans in histopathological evaluation (Fig. 5)

CONCLUSION

The study showed that combination of extracts of *T. copticum, T. foenum* and *N. sativa* in 1:1:1 proportion was effective in controlling the elevated blood glucose levels in STZ induced diabetic rats and also had a po-

tential to reduce the elevated lipid profile in diabetic condition, which could prove beneficial in subsiding other secondary complications of DM.

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