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Study of ameliorating properties of *Tinospora cordifolia* on Diabetes and acute Pancreatitis in Alloxan treated Rats

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ABSTRACT

Diabetes and acute pancreatitis are major health concern globally. India has been growing as a diabetic capital of the world. Diabetes and acute pancreatitis were induced by administration of Alloxan intraperitoneally 150mg/kg.b.wt in to Wistarnorvegicus. Diabetes induction was confirmed by blood glucose concentration above 400mg/dl. The desired plant Tinospora cordifolia (250mg/kg.b.wt) stem extract showed significant remedial action by lowering blood glucose to normal (87mg/dl) and restoring Pancreatic enzymeslipase to (1.74IU/L) and amylase to (583.1IU/L). These results were further authenticated by remodeling of pancreatic histoarchitecture which showed increase in no. Islet of Langerhans, acinar cells, decreased vacuolar space, sinusoidal space and atrophy.

Key words: Alloxan, Acute pancreatitis, *Tinospora cordifolia*, Histoarchitecture

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INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion, insulin action or both [1].

The effect of diabetes mellitus include long term damage, dysfunction and failure of various organs especially eye, kidney, heart and vessels. Typical characteristics symptoms associated with diabetes are thirst, polyuria, blurring of vision, weight loss, polyphagia and in its most severe condition with ketoacidosis or non ketotichyperosmolarity which in the absence of effective treatment leads to stupor, coma and death. Diabetes causes heavy burden on the income and leads to death. Annual reports of WHO 2016 has estimated that 422 million adult aged over 18 were diabetic in 2014.WHO surveyed South East Asia and western pacific region which covers approximately half of the world diabetic population. It was estimated that total death burden till 2012 to be 3.7 million in which 2.2 million deaths from cardiovascular disease, chronic kidney disease and tuberculosis related to higher than optimal blood glucose. It was also mentioned that 43% of all death due to high blood glucose occur before the age of age 70[2]. It is estimated that 35 million in our country already have diabetes and it is expected to reach 70 to 80 million by 2030AD.

Acute pancreatitis is caused due to inflammation of pancreatic gland which may arise due to various reasons like reactive oxidative stress, hypercalaemia, hyperlipidemia, viral infections, some drugs like sulfonamides, steroids etc. However; the exact mechanism behind acute pancreatitis has not been understood yet and due to this there is scarcity of proper treatment.

The present research includes the condition called acute pancreatitis resulting from altered exocrine activity which is the major concern now a day.

Experiments shows 50% prevalence of non pancreatic diabetes 35% in IDDM and NIDDM in case of exocrine abnormality by using direct and indirect pancreatic function test [3]. Serum lipase is of pancreatic origin whose concentration is found to be 5,000 times more than other tissues [4, 5].However lipase is also secreted by stomach, duodenum, liver, heart and tongue [6, 7, 8].

More than threefold elevated level of serum amylase and lipase is found to be the characteristic of acute pancreatitis and meant to be the clinical manifestation used for the diagnosis and its treatment [9, 10].

Intensive use of allopathic medicine causes various life threatening side effects to human body. However treatment by means of natural products is relatively free of side effects and is also cost effective.

Tinospora cordifolia, the desired plant for experiment commonly called as " Guduchi" in Sanskrit and 'Giloy' in hindi belongs to Menispermaceae family[11]. It has a wide range of phytoconstituents which include cordifolide, heptacosanol, clerodanefuranoditerpene, diterpenoidfuranolactone, tinosporine, tinosporide, berberine, magniflorine, choline[12], N- trans- ferulolyl tyramine[13], tinocordiside B,C and D[14]. It has wide range of medicinal properties so it is exploited as a most demanding Ayurvedic medicine for the treatment of allergy, arthiritis, inflammation, lowering of fever, wound, pneumonia, asthma and cough**[15,16].**

Some of the active component isolated from stem of *Tinospora cordifolia* like magnoflorine, syringing, N-formylannonain, cordifolioside, 11-hydroxymustakone and N- methyl -2- pyrolidone enhances immunomodulatory properties, phagocytic activity of macrophages, ROS formation by neutrophil[17,18,19,20,21].It also shows most potent activator of IL-6 cytokines which cures injuries, inflammation, activation of cytotoxic T cell and B cell differentiation [22].In traditional folk medicine it is widely used as hypoglycemic agent which act by promoting insulin secretion, inhibiting gluconeogenesis and by mitigating oxidative stress[23].

MATERIALS AND METHODS

For the present research work wistar rats (*Rattusnorvegicus*) were selected which were obtained from animal market, Tripolia, Patna city, Bihar and breeded up to 3-4 generations in the Biochemistry lab of Patna University to ensure the purity of strain.

Housing

Rats were kept separately in the ratio of two females per male in polypropylene cages of different size Small cage- 26 x 19x13 (h) cms for breeding and Large cage- 40 x 25 x 15 (h) cms for experimentation. Rats were kept in environment that are compatible with life, health and comfort, in such a way that regular needs of the animals, like feeding, cleaning, handling and the turnover of stock could be conveniently met. Water was provided ad libitum

Physical Environment

The temperature of the rat experimentation room was in the range of 24 ^{o} C–28^{o} C. Twelve hours of light and twelve hours of darkness were provided in the room for their optimal growth and reproduction. The light intensity and humidity of the room were maintained at an optimal level.

Feeding

The laboratory rats were fed on laboratory prepared enriched bread constitutes wheat flour, jaggery, powdered milk and gram flour. For providing vitamin supplement they were fed with carrot, sprouted gram and sprouted moong bean.

Details of rats grouping and treatment given to rats

Cage no	Treatment	Average weight	No. of mice each cage	Selected dose (mg/kg b.w)
1	Normal / Control	100-120 gm	5	Normal saline
2	Alloxan treated	100-120 gm	5	150 mg /kg b.wt
3	7daytreated *Tinospora cordifolia*	100-120 gm	5	250 mg/kg b.wt
4	14 days treated *Tinospora cordifolia*	100-120gm	5	250 mg/kg b.wt
5	21 days treated *Tinospora cordifolia*	100-120 gm	5	250 mg/kg b.wt

Preparation of ethanolic extracts of *Tinospora cordifolia* stem (TCS)
The collected leaves of *Tinospora cordifolia* were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by soxhlet's extractor using distilled ethanol as a solvent(90% ethanol) in ratio of 1:5 (100 g powder with 500 ml solvent). The extraction was performed for 18 h. The extract was concentrated by evaporating in vaccum using Rota vapour (Popular, India) at 60^{o}C temperature to ensure minimum denaturation of phytocemicals.

Administration of the extract
Suspension of ethanolic extract was prepared in normal saline. The extracts of *Tinospora cordifolia*(TC) were administered in a dose of 250mg/kg. b.wt, which were selected as per our preliminary studies for its hypoglycemic effect. And Control groups were given Alloxanin normal saline respectively.

Blood collection was done through tail clipping to obtainserum whereas tissue was collected from pancreas after dissection of anaesthesized decapitated rats.

Biochemical assay: For Biochemical analysis was done in Semi-Automatic Chemistry Analyzer (BiosystemBTS-350,Costra Brava 30, 08030, Barcelona).

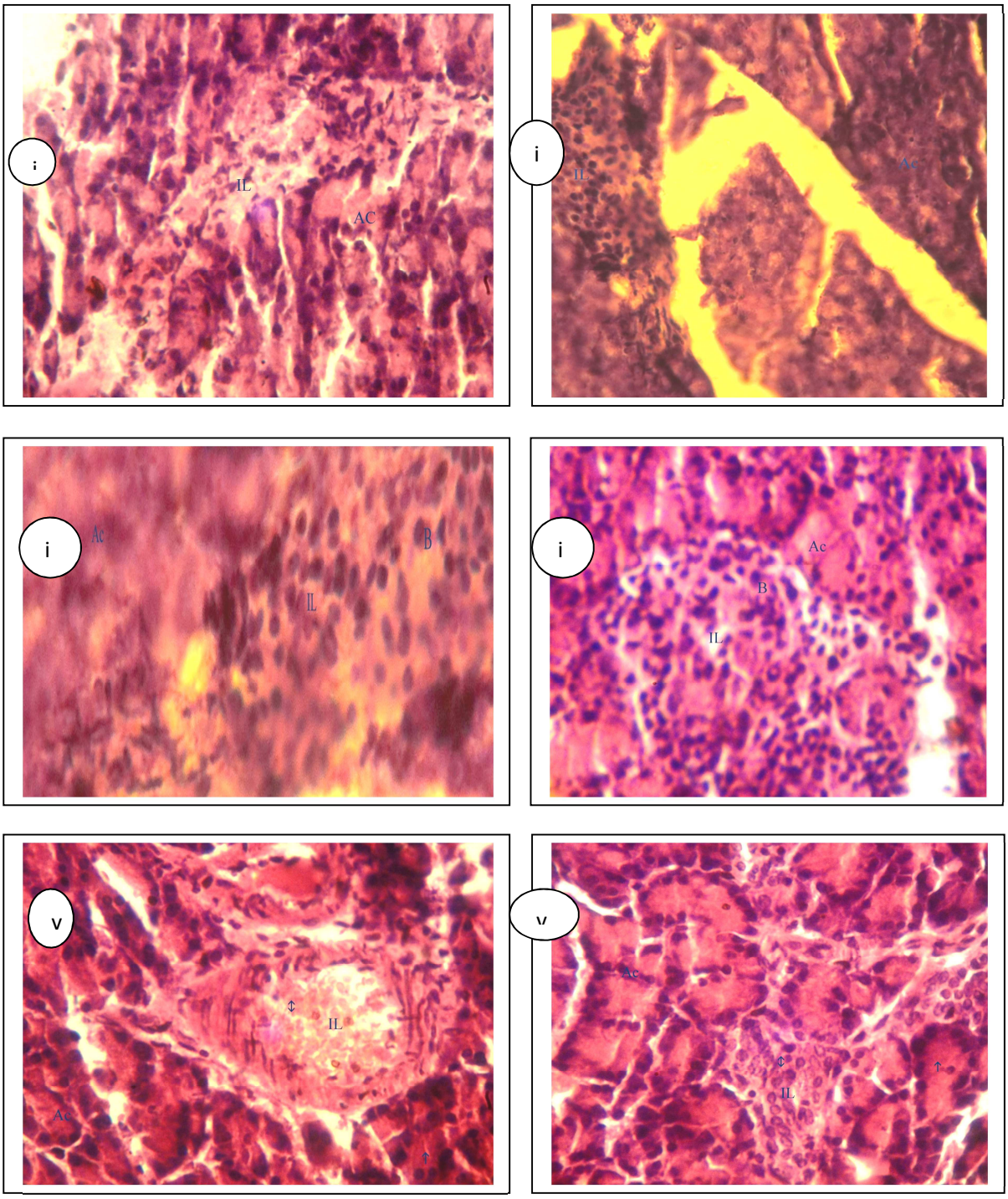
All the biochemical assessments have been done for normal, control and *Tinospora cordifolia* treated rats. 3 observations have been taken in each group.

Glucose test was performed by using Dr. MorpenGluco(Model: BG-03) one blood glucose monitoring system . Amylase was estimated through CNPG3 method obtained from Lifechem, Kamineni Life Sciences, India and lipase by Kinetic colorimetric method obtained from EURO Diagonostic, Spain.

Histopathological assay:
After the rats were sacrificed, Pancreas was immediately separated devoid of fat bodies, chopped and fixed in 10% neutral formaline. Thereafter the tissues were dehydrated in a graded series of alcohol and embedded in paraffin wax. A 5 µm tissue section was cut on microtome, fixed on egg albumin, deparaffinized in xylene and then hydrated descending series of alcohol up to water. Again dehydration was done up to 70% and then stained with Eosin and Hematoxylene and mounted on DPX for microscopic study.

RESULTS

Pancreas plays a very important role as exocrine and endocrine organ. Endocrine role of pancreas is significant in metabolizing glucose which is an important energy source, cells take up the glucose in the presence of insulin to provide energy, in the absence of insulin cells starved and death .In the present experiment alloxan induced diabetic rats has glucose concentration of 600.2±0.158mg/dl, which drastically decreased to 293.2±0.158mg/dl on treatment with *Tinospora cordifolia* on 7thdays. On further treatment with *Tinospora cordifolia* on 14th days decreases to 149.2±0.158mg/dl and on 21st days glucose decreases to 87.4±0.158mg/dl showed hypoglycemic activity of *Tinospora cordifolia.* In the present research work pancreatic function tests like serum amylase and lipase were performed to study exocrine activity along with its histopathological correlation. Normal rats having 600±0.152 IU/L concentration of serum amylase when treated with alloxan has amylase level of 800±0.150IU/L. which on treatment with phytochemical extract restoration was seen ie, 787±0.09IU/L, 725±0.014IU/L and 583.1±0.121IU/L was seen in 7, 14 and 21 days respectively. Simultaneously in case of serum lipase, most potent pancreatic function market similar trend was seen i.e., 1.09±0.08IU/L in case of normal, 6.26±0.120IU/L in case of diabetic but after administration of extract to diabetic rats value significantly declined to 5.11±0.100IU/L, 4.05±0.06IU/L and 1.74±0.112IU/L in consecutive 7, 14 and 21 days.



In this study it was found that both serum amylase and lipase were found to be elevated more than threefold the upper limit shows the condition of acute pancreatitis on exposure to alloxanbut after treatment with *Tinospora cordifolia*phytoextract both serum lipase and amylase were found to be close to normal this result shows both the property of *Tinospora cordifolia*, ie., damage caused in pancreatic acinar cells and regulating the digestive function and mitigating intestinal disorder generally caused in the diabetic condition.

Simultaneously looking towards the histopathological changes we got a positive result regarding repairing of cell damage and its recovery to healthy condition.

Figure 1 shows the normal histoarchitecture of pancreas having normal acinar cells, islets of langerhans and other cells. After treatment with Alloxan 150mg/kg.b.wt intraperitoneally (figure iii and iv) cellular damage had been seen confirmed by tissue degeneration, atrophy, damaged islet of langerhans, widened sinusoids and degenerated acinar cells. Figure v and vi shows phytoextract treated group of 7 and 14 days respectively which confirms regeneration of Islets of Langerhans, Acinar cells, gradual increase in cellular density, decrease in sinusoidal space and atrophy was seen. Figure vi shows pancreatic tissue of 21 days phytoextract treated group which shows therapeutic effect by the recovery of damaged cellular part of pancreas like regeneration of islets of Langerhans, β cells, clear outline of acinar cells with prominent granules, narrowness of tissues space, reduced atrophy and vacuole formation.

DISCUSSION

In the present study Rats demonstrated a significant change in biochemical parameters in terms of blood glucose level, serum amylase, and lipase level as well as in histoarchitecture of pancreatic tissue when exposed to alloxan (150mg/kg.b.wt) followed by treatment with ethanolic extract of *Tinospora cordifolia* (250mg/kg.b.wt) for 21 days at an interval of 7 days respectively. As reported in previous works [24, 25, 26].

In the present investigation administration of alloxan significantly brought destruction of pancreatic tissue which is evident from the increase in fasting glucose concentration compared with the control group. This result is in agreement with [26, 27].

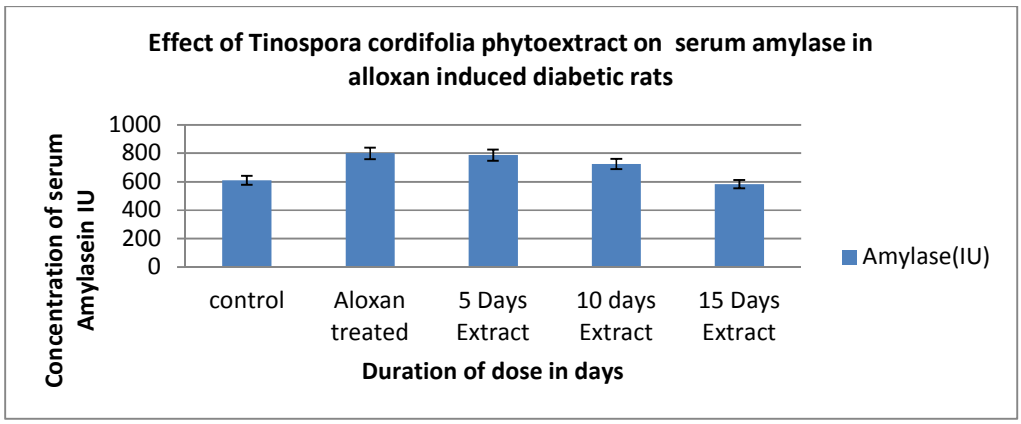
Level of serum amylase and lipase which are secreted from pancreas acinar cell [28] are the important diagonistic marker of acute pancreatitis. In the present work treatment with alloxan caused increase in amylase and lipase indicating pancreatitis effect in accordance with findings of [28, 29, 30]. Similarly Alloxan treatment leads to significant change in histoarchitecture of pancreas as compared to normal which is evident by changes such as vacuolization, necrosis, cellular atrophy and hyperchromic nuclei as reported by other workers [30].

However treatment of rats suffering from diabetes and pancreatitis due to alloxan exposure with ethanolic stem extract of *Tinospora cordifolia* at a dose of 250mg/kg.b.wt significantly restored the blood glucose which is in agreement with previous workers[31,32] indicating the anti hyperglycemic effect of the desired herbal extract [33]. In the present work treatment with *Tinospora cordifolia* extract leads to decrease in level of amylase and lipase level to normal level on 21 days treatment, which may be due to presence of flavonoid[34]. Histopathological studies of pancreatic section of rats treated with ethanolic stem extract of *Tinospora cordifolia* revealed significance amelioration capability to this plane as compared to alloxan treated group which is evidenced by reduction in atrophy, vacuole and necrosis followed by regeneration of acinar cell.

Figure shows (i) Normal rats histoarchitecture having normal cellular arrangement islet of Langerhans (IL) and Acinar cell (Ac). (ii and iii) shows the Alloxan treated Diabetic rats atrophy, tissue degeneration , poor cellularity of islets of langerhans (IL) and damaged islets cells along with hyperchromicity in diabetic control group. Widening of sinusoids(s), necrosis(N) and degenerated Acinar cells (Ac). (iv and v)shows phytoextract treated of 7 and 14 days **.** Increase in the density of cells, decrease in vacoulation, atrophy and sinusoidal space.

(vi) shows section of pancreas showing regenerating islet of langerhans (IL), β-cells (B) and clear outlines of Acinar cell with prominent granules (Ac) in *Tinospora cordifoliaethanolic* extract treated pancreas of rat. Narrowing of tissue space, reduced atrophy and vacuole formation.

TEXT GRAPH-1



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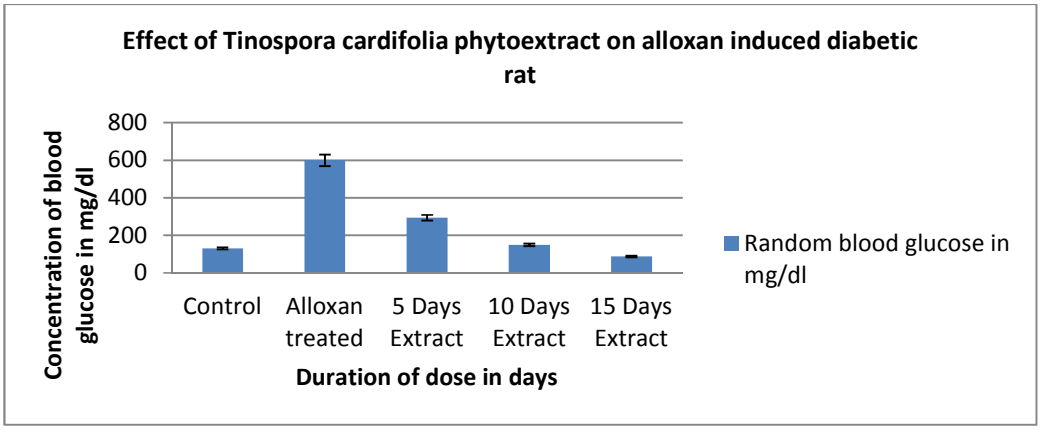

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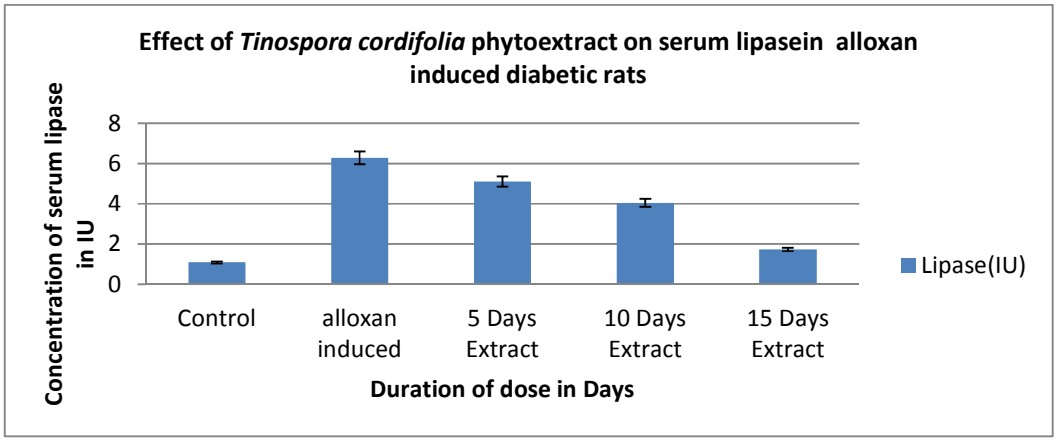

CONCLUSION

The present study showed after treatment with ethanolic extract of *Tinospora cordifolia* for three consecutive weeks restored the damaged histoarchitecture to the normal level which can be justified by the normal blood glucose, lipase and amylase level.

In this way extract pronounces its hypoglycemic effect as well as its remedial exocrine property by restoring lipase and amylase concentration close to normal level. Although study was performed in a limited sample size and parametes but the significant changes in the choosen parameters clearly indicates its prophylactic activity. However further extensive research need to be done to elucidate its potential as therapeutic agent for pancreatitis and mode of action.

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