Hypoglycaemic and Histopathological Evaluation of *Tinospora cardifolia* on Normal and Alloxan- induced Dibetic Rats.

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ABSTRACT

Herbal drugs are prescribed widely as hypoglycemic agents even when their biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low cost. One such plant expected to have hypoglycemic activity is *Tinospora cardifolia* (Menispermaceae). The aqueous extract of stem was studied for hypoglycemic activity in normal glucose loaded and alloxanised albino rats of either sex, weighing 180-200g. Blood samples were collected from tip of tail just prior to 1 and 3 hours after extract administration. During sub-acute treatment, the administration of extract/drug was continued for 14 days, once daily. Blood samples were collected from tip of the tail just prior to and on days 7 and 14 of the extract/drug administration. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method. Animals received extrcat/drug orally at two doses of 250 and 500 mg/kg. In alloxan-induced diabetic rats and normal glucose loaded rats (OGTT) aqueous extract showed statistically highly significant ($P<0.001$) and considerable fall in blood glucose levels. Histopathological slides of pancreas indicated that hematoxylin and eosin sections of pancreas at a dose of 250 mg/kg treated diabetic rats, islet cells at the periphery showed round or ovoid nuclei. However, the pancreas at a dose level of 500 mg/kg treated diabetic rat showed islet cells with normal architecture. The same results were interpreted from hepatocytes.

The present investigation thus justifies use of *Tinospora cardifolia*in traditional system of medicine as an anti diabetic drug.

Keywords: Alloxan, diabetes, *Tinospora cardifolia*, blood glucose

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1. INTRODUCTION

Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels[1]. Diabetes mellitus is a worldwide health problem afflicting millions in both developed and developing countries. It is the primary cause of chronic kidney failure, blindness, high blood pressure and premature coronary artery disease [2]. Diabetes mellitus was known to ancient Indian physicians as 'madumeha'. Many herbal products including several metals and minerals have been described for the cure of diabetes mellitus in ancient literature. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones [3]. Many herbs have been shown to have hypoglycaemic action in animals and humans [4]. In recent years emphasis is on the development of drugs from plants for the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world especially in India [5]. Alloxan diabetic model resembles type1 diabetes (insulin dependent diabetes mellitus) without significant insulin resistance [6]. *Tinospora cardifolia* is one of the herbs mentioned for the promotion and restoration of health and vital force. It is also called Amrita (nectar) in Sanskrit because of its extreme usefulness to man with its properties of strengthening the immune system and keeping the functions of body organs in balance . A necklace, made of small pieces of the stem, known as "Kamlani-Mala" is usually worm in some parts of India as a cure for jaundice. A paste of the plant is plastered over a fractured bone [7].

According to the Ayurvedic System of Medicine, T. Cordifolia is an antigout, analgesic, alterative, reuventator, astringent, anthelmintic, anti-arthritic, antiperiodic, anti-pyretic, anti-malarial, anti-inflammatory, aphrodisiac, antiasthmatic, bitter tonic, carminative, cardiotonic, constipative, digestant, diuretic, blood purifier, expectorant, anti-diabetic, anti-gonorrhoeal, cholagogue, antiemetic and anti-icteric. In current Ayurveda practice, it is used in the treatment of hyperacidity, colic pain, sprue, rheumatism and cellulites. In Sushruta Samhita, it has been described as an antiseptic, febrifuge and curative of oedema, abdominal dropsy, haemoptysis, uro-vaginal disorders. In Charak Samhita, it has been classified as antiagening agent. It is also effective in skin disorders like dermatitis, prickly heat, sun burn, pruritis associated with urticaria, all forms of cosmetic allergy, ringworm, psoriasis, leucoderma and leprosy. It is also effective in plethora of gastro-intestinal disorders like dyspepsia, gastritis, oesophagitis, flatulence, chronic diarrhea, chronic dysentery, hepato-biliary disorders, gastric and duodenal ulcers. In unani system of medicine,it is used an appetiser, stomachic, antipyretic, anti-diabetic, bitter, expectorant, antiemetic, haematinic and blood purifier[9]. As per Charak Samhita, Susruta Samhita, Vagbhata, Sharangdhara Samhita, Vaidyavinoda, Yogaratnakara T. cordifolia roots and stems in combination with other drugs are used as antidote to snake bite and scorpio sting. In Ceylon, stem is used in fever, skin disease and jaundice and syphilis[10]. T. codifolia is found to be effective in cardiac debility, helmithiasis, burnig sensation, erysipelas, uropathy, splenopathy, genral debility seminal weakness[11]. It's decoction with sweet oil is administered in elephantiasis.

Commonly known as Santonica, it is a small deciduous perennial shrub with much branched woody rootstalk, up to 100 cm in height belonging to family Asteraceae. Flower heads when rubbed has a strong agreeable odour. Flowers are yellow in colour while all other parts are whitish grey and has bitter and camphoraceous in taste. The plant is reported to possess hepatoprotective**,** insecticidal, antimicrobial, anthelmintic, cytotoxic and antitumor activity[13]. Traditionally the fresh and dried extracts of top foliage and shoots are given to children against tapeworms and roundworms infestation. It is a bitter aromatic herb or low shrub with much divided leaves and inconspicuous flowers born on numerous small heads. It has been found that no specific work has done on alloxan induced diabetic rats using stem of *Tinospora cardifolia.* So, it was considered worthwhile to investigate the *Tinospora cardifolia* aqueous extract in normal glucose loaded and alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant material

The stems of *Tinospora cardifolia* were collected from Gurdaspur district of Punjab in the month of July. The plant was identified and authenticated by botanist, Dr. Saroj Arora, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar. Its voucher (0400/Hbr) specimen was deposited in the same department for reference[13].

2.2 Preparation of aqueous extract

Shade dried stems all the plants (500 g) were extract with water and filtered. The filtered was dried by vaccum rotary evaeorator which yielded a solid residue of 155 g (31% yield) and was stored at 4^{O}C. The hypoglycaemic effect was evaluated by oral administration of the extract on normal glucose loaded and alloxan induced diabetic rats[15].

2.3 Animals

Adult albino rats of either sex, weighing about 100-150 g were used in the present investigation. Male and female animals were housed separately in groups of 6 per cage (Polycarbonate cage size: 29×22×14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. All the rats were given a period of acclimatization for 15 days before starting the experiment. They were fed *ad libitum* everyday with standard chow diet (HINDUSTAN LEVER, INDIA) and were given free access to water. Animals described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and **S**upervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. SBS/3235).

3. EFFECT OF AQUEOUS EXTRACT OF *TINOSPORA CARDIFOLIA* LINN. ON NORMAL GLUCOSE LOADED RATS

3.1 Effect of test drug on oral glucose tolerance (OGTT)

Fasted normal rats were divided into five groups of six animals each. Group 1 served as control and received vehicle only. Group 2 received the reference drug, glimepride at an oral dose of 150 µg/kg body weight. Group 3 received aqueous extract of stem of *Tinospora cardifolia* (250) at an oral dose of 250 mg/kg body weight. Group 4 received aquous extract of stem of *Tinospora cardifolia* (500) at an oral dose of 500 mg/kg body weight and Group 5 received insulin in the form of injection through i.p route at a dose of 3 I.U/kg. After 30 minutes of extract/drug administration, the rats of all the groups were orally treated with 2 g/kg body weight of glucose. Blood samples (0.2 mL each sample) were collected from the tip of the tail just prior to glucose administration and at 30, and 90 minutes after glucose loading. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method[16].

4. INDUCTION OF EXPERIMENTAL DIABETES

Diabetes was induced by a single ip injection of 120 mg/kg of alloxan monohydrate (S.D Fine-Chem. Ltd., Mumbai, India), in sterile saline. After 72 h of alloxan injection, the diabetic rats were separated and used for the study.

Alloxan monohydrate was dissolved in sterile normal saline immediately before use and was injected intraperitoneally to 18 hours fasted rats at a dose of 150 mg/kg/ bodyweight. After alloxanisation, the animals were given feed *ad libitum* and 5% dextrose solution for the next 24 hrs to overcome initial hypoglycaemic phase due to massive pancreatic insulin release caused by alloxan. The blood glucose levels (BGL) were monitored after alloxanisation in blood samples collected by tail tipping method. The blood was dropped on the detrostix reagent pad and inserted into the microprocessor of digital blood glucometer and reading noted. After 72 hours, rats having BGL beyond 150 mg/dl of blood were selected for the study[17].

5. EFFECT OF AQUEOUS EXTRACT OF *TINOSPORA CARDIFOLIA* STEM ON ALLOXAN INDUCED HYPERGLYCEMIA

5.1 **Acute treatment**

During acute treatment blood samples were collected from the tip of tail just prior to and 1 and 3 h after the extract/drug administration.

5.2 Sub- acute treatment

During sub-acute treatment, all the test samples were administered daily and the treatment period for all these groups was 14 days. Blood samples were withdrawn from the rats by tail vein puncturing with hypodermic needle at 0, 7^{th} and the end of 14^{th} day.

6. STATISTICAL EVALUATION

Evaluation was done by two way- Analysis of Variance (ANOVA) followed by Bonferroni post test. N=6 in each group. P<0.001 and F= 12 (acute) and 122 for (sub acute) which is significant[18].

7. RESULTS

During acute study of the aqueous extract of stem of *Tenospora cardifolia* at different treatments time showed statistically significant reduction in blood glucose level (P<0.001) at 3 hr after alloxan administration (Table 2). During sub acute study, the oral administration ofthe aquous extract of stem of *Tenospora cardifolia* doses of 250 mg/kg and 500 mg/kg body weight reduced significantly (P<0.001) the blood glucose level on 14th day after the administration of alloxan (Table 3).The aquous extract of *Tenospora cardifolia* show the better anti diabetic effect at a dose of 500 mg/kg that showed (49%) of reduction in blood glucose level in comparison to a dose of 250 mg/kg which showed (40%) of reduction in blood glucose level on 14th day after the administration of alloxan[19 and 20]. The oral glucose tolerance test also showed significant decrease (P<0.001) after the treatment at different interval of time with glucose (Table 1).

8. CONCLUSION

The results indicate that aqueous extract of stem of *Tinospora cardifolia* possess significant hypoglycemic activity during both acute and sub-acute treatments. However, the aqueous extract of stem of *Tinospora cardifolia* at a dose of 500 mg/kg body weight showed better efficacy than that with the 250 mg/kg body weight.

9. DISCUSSION

Alloxan produces oxygen radicals in the body, which causes pancreatic injury and could be responsible for increased blood sugar seen in animals. It is generally accepted that alloxan treatment causes permanent destruction of β-cells. It is, therefore, conceivable that the hypoglycemic principles in the aqueous extract of stem of *Tinospora cardifolia* may exert their effect by an extra pancreatic mechanism in diabetic rats. However the exact mechanism is not known and further studies are under investigations.

Table 1: Effect of aqueous extract of stem of *Tinospora cardifolia* on oral glucose tolerance test (OGTT)

S.No.	Groups and doses (mg/kg, b.w)	Blood glucose level (mg/dl)			
		0 min	**30 min**	**90 min**	**% Decrease**
1.	Glucose control	72.0±0.9	250±0.9	140±0.9	---------
2.	Glimepride (150)	70±0.9	145±0.9	78±0.9	79.4%
3.	Stem extract (250)	68±0.9	229±1.3***	135±0.9***	3 .7%
4.	Stem extract (500)	71±0.9	226±1.3***	110±0.9***	27.2%
5.	Insulin (3 I.U/kg)	75±0.9	130±0.9	72±0.9	94.4%

Data expressed as mean ± SEM; N=6. Evaluation by two way- Analysis of Variance (ANOVA) P***<0.001 compared to glucose control; which is significant.

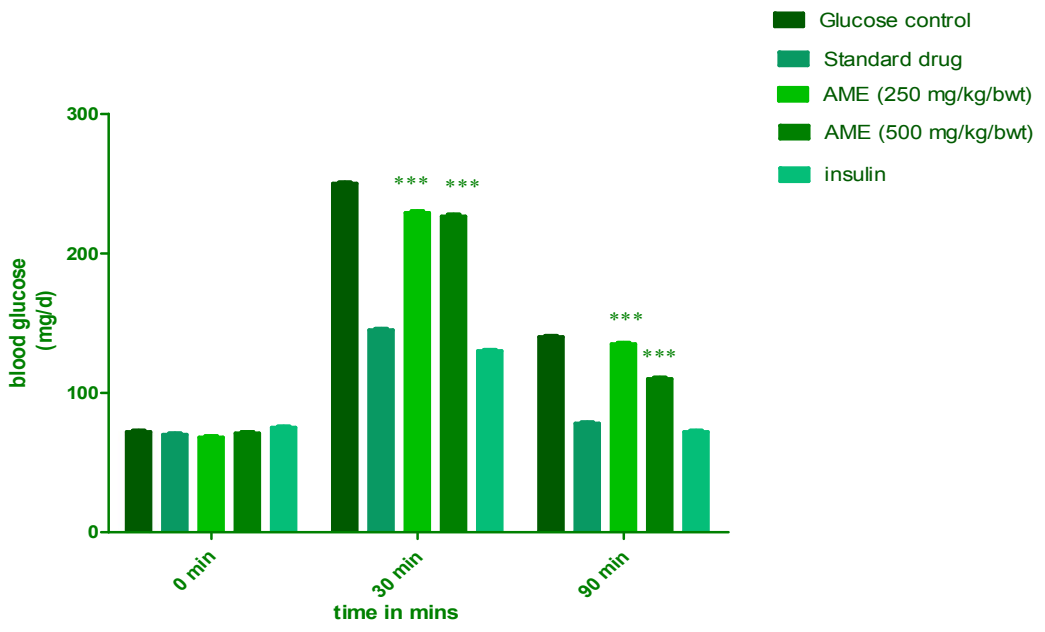

Fig 1: Graph showing the blood glucose level (mg/dl) of different groups of rats on 0, 30 and 90 min after different treatment time.

Table 2: Effect of acute treatment of stem of *Tinospora cardifolia* on blood glucose level in alloxan induced diabetic rats

S.No.	Groups and doses (mg/kg, b.w)	Blood glucose level (mg/dl)			
		0 min	1 hr	3 hr	% Decrease
1.	Normal control	73.3±1.4	75.1±0.8	73.3±0.49	----------
2.	Diabetic control	169±2.6	162±0.7	170±1.3	----------
3.	Glimepride (150)	173±2.4	164±0.8	151±0.9***	12.5%
4.	Stem extract (250)	172±2.2	169±.1.4*	164±0.8***	3.6%
5.	Stem extract (500)	170±4.4	164±0.8	151±0.2***	12.5%
6.	Insulin (3 I.U/kg)	169±3.6	159±1.4	139±1.3	22.03 %

Values are mean ± SEM for six observations N=6: *P<0.05, ***shows P<0.001 compared to diabetic control, which is significant.

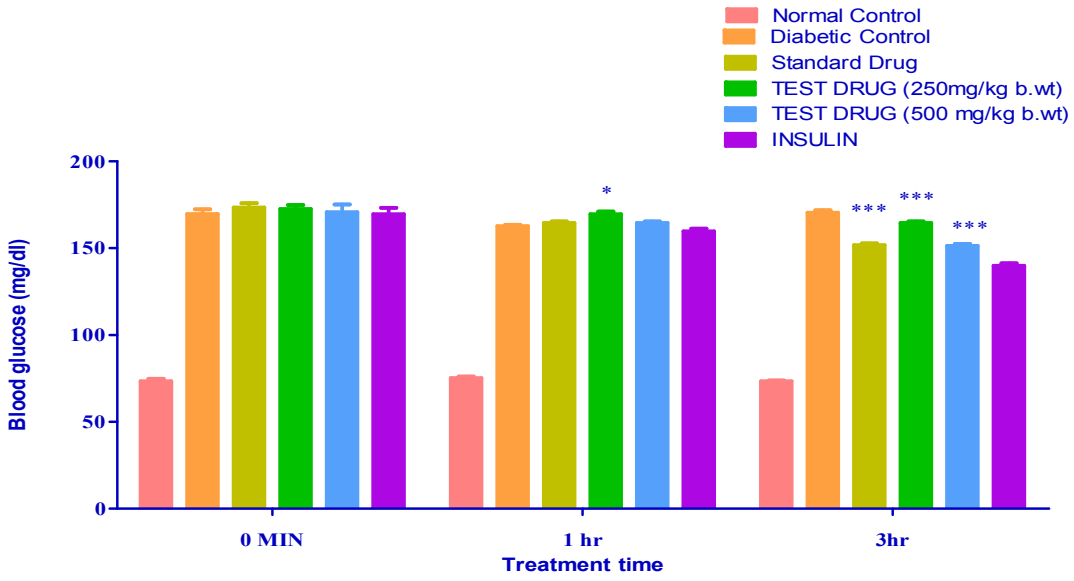

Fig 2: Graph showing the blood glucose levels (mg/dl) of different groups of rats at 0, 1 and 3 hr after different treatment time intervals

Table 3: Effect of sub acute treatment of aqueous extract of *Tinospora cardifolia* on alloxan induced diabetic rats

S.No.	Groups and doses (mg/kg, b.w)	Blood glucose level (mg/dl)			
		0 day	7th day	14th day	% Decrease
1.	Normal control	73.3±1.4	75.3±1.5	76.8±1.3	--------
2.	Diabetic control	169±2.6	197±5.4	230±8.9	--------
3	. Glimepride (150)	173±2.4	80.5±0.7***	73±1.5***	57%
4.	Stem extract (250)	172±2.2	129±2.3***	88.3±2.4***	40%
5.	Stem extract (500)	170±4.4	118±1.5***	81±0.4***	49%
6.	Insulin (3 I.U/kg)	169±3.6	78.8±1.2	73±1.2	57%

Values are mean ± SEM for six observations N=6, ***shows P<0.001 compared to diabetic control.

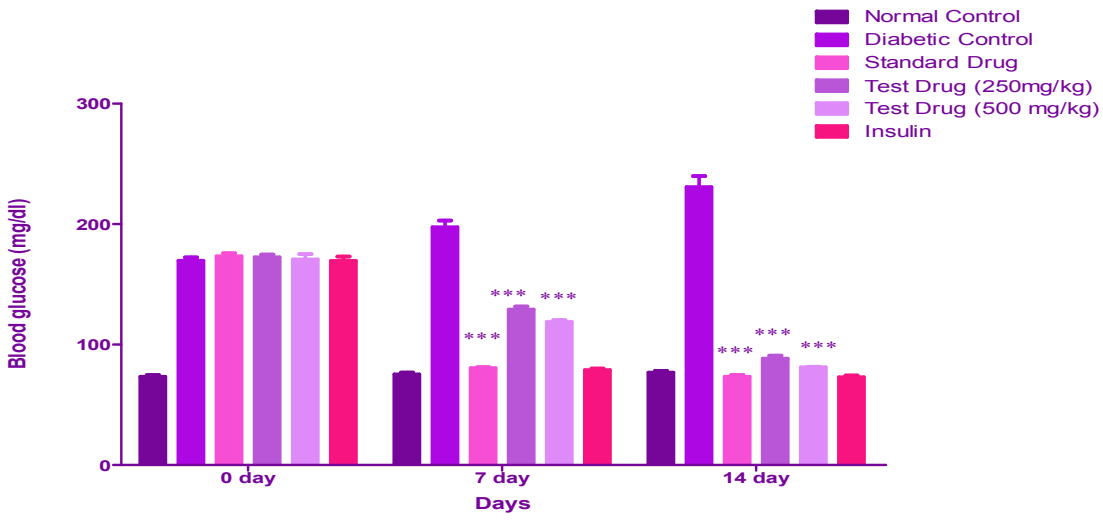

Fig 3: Graph showing the blood glucose levels (mg/dl) of different groups of rats on 0, 7th and 14th days after different treatment time intervals.

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