Antidiabetic, Lipid Lowering and Oxidative Stress Reducing Potential of "*Tinospora cordifolia*" Leaves Powder in Alloxan Induced Diabetic Albino Rats

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ABSTRACT:
Aim: -This study was undertaken to consider the effect of *Tinospora cordifolia* dried leaves powder on glucose level, lipid level and oxidative stress. Analysis was made in curing to anti- diabetic, lipid lowering and oxidative stress reducing properties in albino rats. It can be beneficial to cure type 1 diabetes mellitus.

Methods and materials: - Experimental method was use to analyzed the therapeutic properties of *Tinospora cordifolia* leaves to anti-diabetic, lipid lowering and oxidative stress reducing properties on albino rats. Each group contain six albino rats. Group A was controlled group which was fed with normal pallet rat's diet. Alloxan and ammonium acetate were injected and high fat high cholesterol diet were fed through the diet to the rats everyday for 45day to groups B, C and D. Each group contain six albino rats. *Tinospora cordifolia* dried leaves powder (150 mg/kg body weight) was mixed into the diet and feed them daily. Statistical evaluation was done using mean, standard deviation and student's't' test. 'p' value less than 0.05 was considered statistically significant.

Results: - The results showed significant difference at various levels in anti- diabetic, lipid lowering and oxidative stress reducing properties in albino rats as well as increased level of protein, albumin and haemoglobin in albino rats. No toxicity was found in rats and no side effects were seen.

Conclusion: - *Tinospora cordifolia* dried leaves powder can be used as potential pharmaceuticals in above mentioned diseases. In general *Tinospora cordifolia* leaves are beneficial for disease and its associated problems.

Key Words: - *Tinospora cordifolia,* pharmaceuticals, alloxan, oxidative stress.

I. INTRODUCTION

Widespread use of drugs is leading to the development of resistance against various diseases and pathogen present in it. They have also associated with its side effects influencing people not to use them. The best solution to such problems like diabetes, high cholesterol, viral, malaria, allergy and many more diseases is by traditional method; the herbal medicines, medicinal plants have been used since time immemorial for the treatment of uncountable diseases. Usually ayurvedic drugs are being used due to their minimum toxicity. Diabetes mellitus is a syndrome, associated with hyperglycemia, hyperlipidemia, oxidative stress, polyurea, polyphagia, polydypsia, ketosis, nephropathy, neuropathy and cardiovascular disorders [1]. In modern medicine no satisfactory effective therapy is yet available to cure diabetes mellitus. Although, insulin therapy is used for managing of glucose level but on the other hand there are several drawback like insulin resistance anorexia nervosa, brain atrophy and fatty liver. Its proper handling is also required for refrigeration of insulin and skilled technician even these are higher in costs which are not reasonable for poor people. Although iron is ample amount on soil but iron insufficiency is very ordinary in humans and is the most widespread root of anemia globally. To more fully understand iron deficiency anemia, consideration must be directed toward concepts of iron supply and demand for the production of erythrocytes. So, herbal medicines are cheaper in costs and also easily available in surroundings.

Tinospora cordifolia (family Menispermacea; commonly known as Guduchi or Giloy), a glabrous climbing shrub, is widely used in folk and ayurvedic system of medicine in India since ancient times. The whole plant is used for therapeutic purpose. It is reported that the plant is bitter in taste but nontoxic and also has ability to scavenge free radicals. Leaves of this plant are rich in protein and are fairly rich in calcium and phosphorus [2]. *Tinospora cordifolia* has been used to treat general weakness, fever [3], dyspepsia [4], dysentery, gonorrhoea [5], secondary syphilis, urinary diseases [6], impotency [7], gout [8], viral hepatitis [9], skin diseases and anaemia [10-11]. Its remarkable and notable medicinal properties such as anti-diabetic [12], anti-periodic[13], anti-spasmodic[14], anti-malarial[15], anti-flammatory[16], anti-arthritic[17], anti-oxidant[18], anti-allergic[19], anti-stress[20], anti-leprotic[21], hepatoprotective [22], immunomodulatory [23], blood purification and anti-neoplastic activities [24] are well documented[25-26].

II. MATERIALS AND METHODS

The current study was carried out in Department of Food Science and Nutrition at Banasthali University, Distt- Tonk, Rajasthan, India after approval by Institutional Animal Ethics Committee. Handling and care of animals was according to CPCSEA guidelines. Care was taken during the animal study including food, water and shelter place (Housing) to prevention from infection.

2.1Collection of plant materials

Leaves of *Tinospora cordifolia* were collected from different places of Banasthali Vidhypith campus, Rajasthan, India.

2.2Preparation of leaves powder

Tinospora cordifolia fresh leaves were washed under the running tap water and then dried under the shade at room temperature. Dried leaves were powdered in electronic grinder and stored in air tight container for further use.

(a) Fresh Leaf **(b) Dried Leaf**

Figure 1. (a) fresh leaf of giloy, (b) dried leaf of giloy,

2.3Pre- Supplementation Phase

2.3.1 Animals

Twenty four male albino rats (wistar addendum strain), 4-5 weeks old and weighing 100-150 gm were procured from Haryana Agricultural University, Hisar. In the pre experimental period of a fortnight, the animals were given free access to standard rat pellet diet and water.

2.3.2 Experimental Design

After initial period of adaptation, the animals were divided into four groups in such a way that average weight of each group constituting six rats remained almost similar. Rats were housed in solid bottom plastic cages with stainless wired top. Each group was fed on different types of diet. The temperature of the animal room was maintained between 20-25˚C and weekly weight was monitored. Actual consumption of diet of every rat was determined by weighing food given as well as left over's every day.

2.3.2.1 Composition of Diet

The diet for the rats comprised of standard pellets procured from Ashirwad diet, Chandigarh with the following composition:

Table No.-1: Control group diet:

Nutrients	Quantity(g/kg)
Carbohydrates	300
Protein	100
Fat	25
Crude fibre	25
Ash	45

2.3.2.1 Experimental Group Diet

The animals in the high fat groups were administered High-Fat High-Cholesterol diet. However, both the diets were made isocaloric at the expense of carbohydrate. Casein was used as a source of protein and coconut oil and refined oil (groundnut oil) combination was used to provide fat. The mineral and vitamin mixes were prepared to provide essential and potentially beneficial mineral elements. Composition of experimental diet has been mentioned in the table below:

Table No.-2: Composition of experimental diet

S. No.	Ingredients	Quantity (g/kg)	S. No.	Ingredients	Quantity (g/kg)
1	Carbohydrates	150	9	Crude fibre	160
2	Protein	50	10	Ash	45
3	Fat	12.5	11	Carbohydrates (starch)	50
4	Crude fibre	12.5	12	Sucrose	20
5	Ash	22.5	13	Cholesterol	5
6	Casein	100	14	Bile Salts	1.25
7	Fat (Refined oil)	3.5	15	Folic acid	0.165
8	Coconut oil	135.4	16	Multivitamin	1.44

2.3.3 Drugs used in the study

2.3.3.1 Alloxan

Alloxan was induced to B and D group alloxon monohydrate dissolved in normal saline at a dose of 120mg/kg body weight.

2.3.3.2 Ammonium acetate

Oxidative stress is known as chemical trigger free radical information and thus was used to see the effect of bioactive principle on the oxidative stress status of rats in a short period of time. Oxidative stress was induced to C and D the groups by ammonium acetate at a rate of 125mg/kg body weight.

2.3.3.3 High-Fat High-Cholesterol diet (HFHC)

However, the diets were made isocaloric at the expense of carbohydrate. Casein was used as a source of protein and coconut oil and refined oil (groundnut oil) combination was used to provide fat.

The mineral and vitamin mixes were prepared to provide essential and potentially beneficial mineral elements with water.

Group A- Control: Isoenergic Normal Fat Diet

Group B- Normal Fat Diet +Alloxan

Group C- High Fat and High Cholesterol diet + Ammonium acetate

Group D- High Fat High Cholesterol diet + Alloxan + Ammonium acetate

\+ *Tinospora cordifolia* leaves powder (150g/kg body weight)

2.4 Post Supplementation Phase

At the end of 45 days experimental period, food was with hold overnight. The rats were anaesthetized with diethyleather the following morning and blood withdrawn from the orbital sinus into tubes. Thereafter, the animals were sacrificed by cervical dislocation. The liver, kidney, heart and brain were removed and washed with ice cold saline solution, weighed and immediately processed for biochemical analysis.

Collection of blood for preparation of Serum, Lipid Extract from Liver Tissue of rats [27], Post Mitochondrial Supernatant (PMS) and Brain Homogenate were also prepared for further analysis.

2.4.1 Biochemical Analysis of Blood/Serum

Estimation of haemoglobin by Sahli's method [28], glucose [29], total cholesterol [30], high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) [31], albumin [32], protein [33], triglycerides [34], uric acid [35], SGOT (Serum Glutamate Oxaloacetate Transaminase) [36], SGPT (Serum Glutamate Pyruvate Transaminase) [36] on serum were also analysed.

2.4.2 Biochemical Analysis on Liver Homogenate

Free fatty acids (FFA) [37], triglycerides (TG) [38], total cholesterol (TC) [39], phospholipids [40] were also analysed on liver tissue.

2.4.3 Biochemical Analysis of Post Mitochondrial Supernatant (PMS)

Estimation of Reduced Glutathione [41], Glutathione Peroxidase [42] and Superoxide dismutase [43] were determined on brain homogenate.

2.5 Statistical Analysis of Data

The statistical methods used for the analysis of data for the present study were: - Mean, Standard deviation, T- test. If p value is < 0.05 then it was considered statistically significant (s) and if not it was considered statistically non-significant (ns).

III. RESULTS

3.1 Body Weight and Food Consumption

Body weight was decreased significantly in alloxan induced diabetic group (B) as compared to other groups (A), (C) and (D). There is no significant difference in body weight of normal group (A) and group treated by *Tinospora cordifolia* leaves powder (D) (Table 3). HFHC diet showed significantly increased the mean relative food consumption in normal fat fed rats to HFHC fed diet ones but significantly decreased in diabetic group. However, concomitant consumption of HFHC *Tinospora cordifolia* leaves powder significantly reduced the fattening effect of high fat diet (Table 3). Research showed that increased food consumption and decrease body weight observed in diabetic control group (B) in comparison to normal group (A) indicates polyphagia condition and loss of weight due to excessive breakdown of tissue proteins. Treatment with *Tinospora cordifolia* leaves powder group (D) food consumption and improved body weight to some extent indicating control over polyphagia and muscle wasting resulted due to hyperglycaemic conditions.

Table 3 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder on body weight of albino rats.

Dietary Groups	Initial Weight (gm)	Final Weight (gm)	Food Consumption (gm)
GROUP A	100.8±0.8	114.8±9.7	6.8±0.51
GROUP B	110.3±8.7	100.5 ± 6.2^{s}	6.0 ± 0.49^{s}
GROUP C	108.83±5.7	125.3 ± 8.2^{s}	6.5 ± 0.22^{ns}
GROUP D	121.66±17.2	135.8 ± 15.7^{ns}	6.4 ± 0.47^{ns}

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

3.2 Organ Weight

Net increase in organ weight of animals partaking HFHC diet and HFHC *Tinospora cordifolia* leaves powder diet over that of control group fed Isoenergic normal fat diet (Table 4). Liver, kidney, heart and brain weight are lower in diabetic group (B) as compared to other groups (A), (C) and (D).

Table 4 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder on different organs weight of albino rats.

Dietary Groups	Liver Weight (gm)	Kidney Weight (gm)	Heart Weight (gm)	Brain Weight (gm)
GROUP A	6.7±0.42	1.2±0.19	0.6±0.01	1.3±0.20
GROUP B	5.7 ± 0.37^{s}	1.0 ± 0.22^{s}	0.4 ± 0.02^{s}	1.0 ± 0.30^{s}
GROUP C	6.9 ± 0.97^{ns}	1.3 ± 0.20^{ns}	0.5 ± 0.01^{ns}	1.2 ± 0.19^{ns}
GROUP D	7.3 ± 0.65^{ns}	1.4 ± 0.39^{ns}	0.7 ± 0.04^{ns}	1.5 ± 0.19^{ns}

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

3.3 Blood Glucose Status

The effect of HFHC of *Tinospora cordifolia* leaves powder diet group (D) on blood glucose status of albino rats has been depicted (Table 5). The means blood glucose of normal fat diet diabetic rats group (B) increased significantly from rats reared on HFHC diet group (C). Simultaneous feeding of *Tinospora cordifolia* leaves powder (D) significantly reduced the mean blood glucose level in both the groups (B) and (C).

Table 5 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder diet on blood glucose status of albino rats.

Dietary Groups	Glucose Fasting Blood Sugar (mg/dl)		Glucose Post Prandial (mg/dl)	
	Pre Supplementation	Post Supplementation	Pre Supplementation	Post Supplementation
GROUP A	102±5.4	107±5.1	120±5.3	127±5.0
GROUP B	104.6±7.3	159.6±6.5^{s}	123±4.9	183±4.1^{s}
GROUP C	107.3±5.3	143.3±5.3^{s}	121±2.7	157±3.7^{s}
GROUP D	106.9±7.2	116.5±6.4^{ns}	120±5.5	135±4.6^{ns}

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

The Fasting blood glucose concentrations of diabetic rats were significantly lowered with the plant therapy. In this therapy *Tinospora cordifolia* leaves powder fed diabetic showed significantly decreased in blood glucose as compared to diabetic group (B) and HFHC group (C) (Table 5).

3.4 Serum Lipid Profile

Effects of HFHC diet and its modification by incorporation *Tinospora cordifolia* leaves powder on serum lipid status of albino rats were evaluated. Results revealed that HFHC diets resulted in hypocholesterolemic condition in group (C) rats (Table 6). Group concomitant feeding of *Tinospora cordifolia* leaves powder (D) resulted in triglycerides marked decrease in total cholesterol and other lipoprotein fractions. Study showed that there is fall in serum concentration of cholesterol, triglycerides, and LDL- cholesterol in group (D) diabetic rats. A significant increase in HDL- cholesterol level was also seen in the same group.

Table 6 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder diet on serum lipid profile of albino rats.

Dietary Groups	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
GROUP A	87±2.6	161.17±3.5	40.77±3.5	105.3±1.6
GROUP B	100.8±7.4^{s}	185.87±3.7^{s}	33.07±2.0^{s}	128.0±7.3^{s}
GROUP C	107±5.3^{s}	200.50±2.4^{s}	34.3±1.4^{s}	139.4±8.3^{s}
GROUP D	90.28±3.6^{ns}	170.5±6.3^{ns}	40.1±2.92^{ns}	110.5±3.5^{ns}

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

3.5 Albumin, Protein and Haemoglobin Levels

(Table 7) Showed that there was increased in serum concentration of albumin and protein levels of *Tinospora cordifolia* leaves powder fed diabetic rats i.e.; group (D) then the other two groups (B) and (C). There was increased level noticed in haemoglobin of blood concentration of *Tinospora cordifolia* leaves powder fed diabetic rats then the other three groups (A), (B) and (C).

Table 7 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder diet on albumin and protein levels of albino rats.

Dietary Groups	Albumin (g/dl)	Protein (g/dl)	Hemoglobin Concentration (g/dl)
GROUP A	4.3±0.5	7.2±0.7	13.4±0.9

GROUP B	2.6±0.4^{s}	4.5±0.3^{s}	9.8±2.6
GROUP C	2.1±0.5^{s}	4.3±0.6^{s}	10.2±0.8
GROUP D	3.9±0.5^{ns}	6.3±0.3^{ns}	16.2±1.9

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

3.6 SGPT, SGOT and Uric Acid Levels

Study showed that there is serum fall in concentration of SGPT, SGOT and uric acid level in *Tinospora cordifolia* leaves powder fed diabetic group (D) then the control diabetic group (B) and HFHC fed group (C). There is a significant decrease in serum uric acid is also seen between these groups (Table 8).

Table 8 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder diet on serum transaminease and uric acid levels of albino rats.

Dietary Group	Serum SGOT (IU/L)	Serum SGPT (IU/L)	Uric acid (mg/dl)
GROUP A	49.8±0.6	27.8±0.6	4.4±0.4
GROUP B	94.9±0.6^{s}	44.9±0.6^{s}	6.5±0.1^{s}
GROUP C	85.6±0.8^{s}	46.6±0.8^{s}	6.3±0.4^{s}
GROUP D	50.4±0.8^{ns}	34.6±0.7^{ns}	5.0±0.6^{ns}

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

3.7 Hepatic Lipid Status

The effect of HFHC diet (C) and HFHC *Tinospora cordifolia* leaves powder diet (D) on the hepatic lipid status of albino rats has been depicted in (Table 9). In contrast, group (D) showed a marked decrease hepatic lipid levels to group (B) and (C) respectively. Study also showed that there is fall in concentration of Phospholipids, Triglycerides, Total Cholesterol and Free Fatty acid in liver tissue of HFHC *Tinospora cordifolia* leaves powder fed diabetic rats (Table 9).

Table 9 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder diet on hepatic lipid status of albino rats.

Dietary Group	Phospholipids (mg/dl)	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	Free Fatty acid (mg/dl)
GROUP A	117.1±4.6	417.5±1.7	368.8±5.2	590.5± 0.04
GROUP B	153.8±7.6^{s}	444.1±7.0^{s}	428.2±4.4^{s}	684.7±0.0^{s}
GROUP C	158.5±5.3^{s}	489.0±2.9^{s}	371.0±5.4^{s}	697.8±0.0^{s}
GROUP D	126.1±4.7^{ns}	424.1±1.6^{ns}	359.2±3.7^{ns}	595.6±0.0^{ns}

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

3.8 Oxidative Stress

Blood serum and hepatic tissue determination were made to assess the oxidative stress status, which include superoxide dismutase, reduced glutathione and glutathione peroxidase activity. As described in the (Table 10) the enzyme activities were significantly increased in control diabetic group and HFHC fed group but HFHC *Tinospora cordifolia* leaves powder supplemented with reduced the mean scores significantly.

There was marked rise in superoxide dismutase observed in controlled diabetic rats. Whereas, significant decrease in glutathione peroxidase of *Tinospora cordifolia* leaves powder fed diabetic rats were seen. In the same way slightly decreased activity of reduced glutathione were observed in controlled diabetic rats (B) and HFHC fed rats (C) and levels were significantly regulated in *Tinospora cordifolia* leaves powder therapy indicating modulation over brain oxidative stress (Table 10).

Table 10 - Effect of HFHC diet and HFHC with "*Tinospora cordifolia*" leaves powder diet on brain oxidative stress of albino rats.

Dietary Groups	Reduced Glutathione (nm/100ml)	Superoxide dismutase (SOD) (nm/100ml)	Glutathione Peroxidase (nm/100ml)
GROUP A	353.7±1.0	2.2±0.7	8.4±0.3
GROUP B	283.8 ± 5.8^{s}	3.1 ± 0.4^{s}	6.0 ± 0.4^{s}
GROUP C	268.0 ± 8.0^{s}	3.8 ± 0.2^{s}	6.2 ± 0.3^{s}
GROUP D	333.0 ± 7.89^{ns}	2.5 ± 0.3^{ns}	7.8 ± 0.3^{ns}

Group A was compared to group B, C and D and respectively the level of significance was checked at 0.05 levels; significant (s), non-significant (ns).

IV. DISSUCISSION

Uses of herbal plants are there since ancient times but they are more in limelight these days. *Tinospora cordifolia* is one of the famous climbers used for treating numerous diseases. Richest source of nutrients and phytochemicals its all parts are used as medicine for animals as well as humans. *Tinospora cordifolia* dried leaves powder successfully showed the positive effects against the glucose, lipid and oxidative stress levels as we are seen in the tables and results. Supplementation of leaves powder of plant resulted in a significant correction of blood glucose level with respect to alloxan induced diabetic HFHC fed group. Other research also showed positive results curing diabetes when treated by *Tinospora cordifolia* [14, 17-18, 25, 44, 45]. Following the leaves powder supplementation in diabetic HFHC fed rats there was a significant recovery in hepatic lipid level also the same result showed in [46]. The control levels of oxidative stress where the degree of recovery was more significant after leaves powder enhancement in the diet of group (D) same results in [47-49]. These recoveries were more effective when treatment of composite of leaves powder was used which primarily focuses on the anti-diabetic, anti-hyperlipidemic and anti-oxidative stress activity of the plant leaves powder. Hyperlipidemia associated with hyperglycemia, and oxidative stress leads to the amplified levels of glucose, SOD, reduced glutathione and glutathione peroxidise. Due to presence of phytochemical it showed protection in opposition to aflatoxins [50-53]. After the supplementation of leaves powder the levels were near about the normal levels. This leads that *Tinospora cordifolia* can be used in curing potential pharmaceuticals for diabetics, hyperlipidemic and oxidative stress patients and many diseases conditions.

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