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Comparative Hypoglycemic Effects of Different Extract of Clitoria Ternatea Leaves on Rats

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Abstract: *We evaluated the hypoglycemic effects of methanol, water, petroleum ether, chloroform extract of Clitoria ternatea leaves. The hypoglycemic effect was evaluated in Streptozotocin induced diabetic rats for acute and sub acute effects. The extract of Clitoria ternatea also significantly reduced blood glucose level in Streptozotocin induced diabetic rats.*

Keywords: *Hypoglycemic, Clitoria ternatea, streptozocin, antidiabetic activity.*

I. Introduction

Diabetes mellitus (DM) is a widespread disorder, which has long been recognized in the history of medicine, before the advent of insulin and oral hypoglycemic drugs, the major form of treatment involved the use of paints. More than 400 plants are known to have been recommended ad recent investigations have affirmed the potential value of some of these treatments.

Diabetes mellitus is a group of endocrine syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications from vascular disease. Most patients can be classified clinically as having either type I diabetes mellitus (type I DM formerly known as insulin dependent diabetes of IDDM) or type II diabetes mellitus (type II DM formerly known as non-insulin dependent diabetes of NIDDM). A good-looking perennial twining herb with terete stems and branches, leaves compound, imparipinnate, leaflets 5-7, sub- coriaceous, elliptic- oblong, obtuse; flowers blue or white , solitary axillary or in fascicles, corolla papilionacaous; fruits nearly straight, Flattened pods, Sharply beaked; seeds 6-10, smooth, yellowish brown. Clitoria ternatea is used as aphrodisiac tonic and are useful in ophthalmopathy. The leaves are useful in otalgia, hepatopathy and eruptions. The root also has anti-inflammatory, analgesic and antipyretic properties. Clitoria ternatea is used in leucoderma, burning sensation and pains. The roots are used as bitter, refrigerant, ophthalmic and laxative.

II. Material And Method

Plant material: The fresh leaves of Clitoria ternatea was collected during the month of September 2011, from the pratap Nursury, karamchari nagar bareilly . The plant materials was taxonomically identified and authenticated by Dr.umesh chand pandey , HOD and in charge Botany Department, Bareilly college, bareilly**BCB/BOT /376/24-01-2012.**

Preparation of Extract. The leaves of Clitoria ternatea were shaded dried until cracking sound was observed during breakage, and then these are made into coarsely powdered from using dry grinder. The powder leaves of the plant (500 gm) was macerated with each different solvents methanol, water, chloroform, petroleum ether (1500 ml) at room temperature for 72 hours with occasionally stirring. The extracts were separated from the residues by filtering 1st through several layers of muslin cloth for coarse filtration and then through what man No. 1 filter paper. The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness at temperature not exceeding 40^0C and then give moderate heating on water bath at temp 40±5 ^{o}C. The extracts were kept indifferent Petri dish and it was stored in refrigerator (-4c) at cool place till use. During experiment the crude extracts were diluted (100 mg of the extract was dissolved in 0.5 ml water) with distilled water just before administration to the animal.

Animals: Male swiss albino mice of body 150-200 gm weight were taken before and after experiment with the help of single pan balance were used for the study. The animals were housed in clean metabolic cages and maintained in controlled temperature (27± 2^0C) and light cycle (12 hrs. light and 12 hrs. dark). They were fed with standard pellet diet (Gold mohar brand, Lipton India Ltd.) and water. The protocol was approved by Institutional animal ethics committee. (1452//PO/a/11/CPCSEA).

Streptozotocin: Streptozotocin (STZ) is a naturally occurring nitrosourea product of Streptomyces achromogenes. Usually, the intraperitoneal injection of a single dose (25 mg/kg body weight) of it exerts direct

toxicity on β cells resulting in necrosis within 48-72 h and causes a permanent hyperglycemia. STZ breaks nuclear DNA strand of the islet cells.

Preparation of Dose: The Dose of 200 mg/kg and 400 mg/kg of all extract was selected for the test. All the doses was given orally after making emulsion in vehicle i.e. 1% acacia gum and the standard drug i.e. glibenclamide was given orally (10 mg/kg) in the vehicle.

III. Experimental Work:

1. Effect of different extract on streptozotocin induced diabetic rats:

A. Induction of diabetes

Streptozotocin manufactured by Sisco Research laboratories Pvt. Ltd. Mumbai, India and was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 25 mg/kg body weight and injected intraperitoneally within 15 min of dissolution in a vehicle volume of 0.4 ml with 1 ml of tuberculin syringe fitted with 24 gauge needle, where as normal control group was given citrate buffer only (0.4 ml). Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin.

B) Sample collection: Blood sample were collected from tail nipping and glucose level was determined by an automatic electronic glucometer (Accuchek comfort).

C) Procedure: Each group consisted of 8 mice in the beginning of the study. After checking the fasting blood glucose in overnight fasted rats. They were divided into five groups of five rats each and one group of non-diabetic rats.

All the doses were given in the following manner

- 1st Group- normal control group received vehicle.
- 2nd group was treated as diabetes control group.
- 3rd Group-Received each extract at dose of 200 mg/Kg orally.
- 4th Group- Received each extract at dose of 400 mg/Kg. orally.
- 5th Group- Received standard drug i.e. Glibenclamide (10 mg /Kg. in Vehicle) orally.

The treatment was continued for 4 hours. During the period water was supplied ad libitum. All the doses were administered orally by the oral feeding needle. The effect of extract on Blood glucose levels was estimated on overnight fasted rats on 0 hour, 30 min, 60min, 120min and 240min by the method described before. The general behaviors of the animals were recorded. The blood glucose level in (Mean ± SEM) is shown in the Table.

IV. Result And Discussion

The methanol extract have the very good effect on the blood glucose level after given glucose the blood glucose level was recorded standard drug have the maximum effect but the methanol extract of 400mg/kg also have a significant effect. 200 mg/kg decreased glucose level but not as 400mg/kg as compare to the control group. According to it the 400mg/kg would be useful in the diabetes to control the increased glucose level.

As the result of acute effect of the methanol extract shows that 200mg/kg and 400 both has the very similar effect but after 240 minutes the 400mg/kg shows minimum blood glucose but at the initial stage at the 30 min 200mg/kg shows a fine decrease in blood glucose level.

The sub acute effect of the methanol extract reported that the first day the group 3rd found lowest blood glucose level and also at the final day of the study the result was same that group 3rd showed minimum glucose level it shows that on the long term use of 200mg/kg has more potential than the 400mg/kg. The effect of 200mg/kg of methanol extract was not better than standard drug but was good enough. Diabetic control shows highest blood glucose level .on the other hand standard drug shows the best result among the all extract. But methanol dose 400mg/kg shows prominent result. Chloroform shows minimum effect on the blood glucose level. In the 200mg/kg methanol also shows good effect but these was not good than the 400 mg/kg of the methanol extract.

The result of the sub acute activity is much differs than the acute study this result shows that on the long term use of extract the dose 200 mg/kg is much better to control the blood glucose level tan the 400 mg/kg dose. The effect of the standard drug and different extract the methanol extract of 200 mg/kg dose showed 15 gm in the fifteen days and standard drug showed 10 gm

weight gain in the animal. But in the case of chloroform extract there is something different because it showed 7gm weight loss in the 200 mg/kg dose. Water 400 mg/kg, 200 mg/kg showed within 3gm ± weight difference in the animal. Petroleum ether 400 mg/kg also showed 7gm weight loss in the animal during 15 days study. Diabetic control group showed 3gm weight gain.

Table 1: Anti diabetic effects of different extract of clitorea ternetea leaves on glucose loaded rats

GROUP	0 min	30 min	60 min	120 min	240 min
control	75.92 ±2.21	177.50 ±4.38	151.89 ±3.54	126.32 ±3.61	105.67 ±2.76
Standard	81.85 ±2.52	147.01 ±2.00	119.81 ±2.86	86.97 ±3.03	82.34 ±2.13
Methanol 200	71.52 ±1.37	159.50 ±3.73	135.68 ±2.10	110.37 ±1.64	96.49 ±4.23
Methanol 400	77.30 ±3.07	153.40 ±2.52	130.73 ±2.38	101.74 ±1.60	91.54 ±3.29
Water 200	72.46 ±2.32	162.52 ±3.72	139.23 ±2.31	109.64 ±2.57	98.26 ± 3.13
Water 400	76.42 ±2.34	155.76 ±3.12	132.54 ±3.76	197.35 ±2.19	94.87 ± 2.31
Chloroform200	74.24 ±4.34	170.22 ±2.78	161.39 ±3.40	130.34 ±5.15	117.40 ± 3.73
Chloroform400	72.46 ±3.34	168.12 ±2.32	164.65 ±4.52	135.78 ±3.37	112.20 ± 3.82
Pet ether 200	7 1.44 ±3.37	165.34 ±2.49	144.53 ±3.32	119.98 ±3.45	101.87 ±2.56
Pet ether 400	74.47 ±5.90	160.41 ±2.89	142.65 ±4.52	130.78 ±3.27	107.63 ±4.40

Table 2: The comparative Antihyperglycemic effect of different ExtractS on STZ induced Diabetic rats

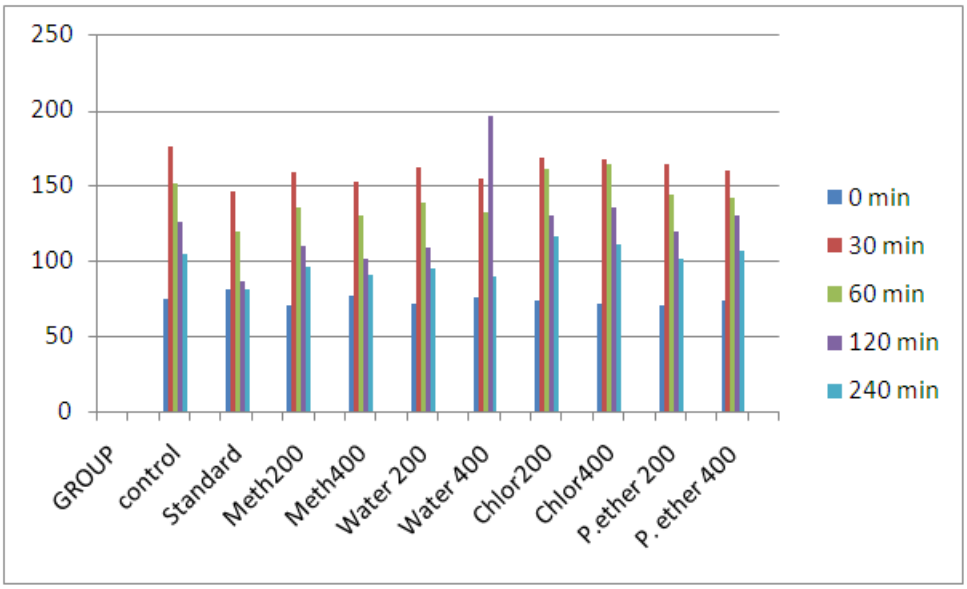
	Blood Glucose Level (mg/dl) at hr				
GROUP	**0 min**	**30min**	**60 min**	**120 min**	**240 min**
control	75.75 ±3.93	75.67 ±2.75	75.56 ±2.20	76.63 ±1.59	76.06 ±1.48
Diabetic control	343.37 ±8.04	342.19 ±6.37	340.52 ±5.48	333.69 ±4.57	325.54 ±4.39
Methanol 200	332.67 ±3.51	292.56± 3.72	290.48 ±3.56	264.92 ±2.23	254.19 ±3.40
Methanol 400	342.32 ±3.12	308.12± 2.30	287.41 ±3.32	266.38 ±2.43	250.19 ±2.43
Water 200	340.82 ±4.51	298.45 ± 3.27	289.95 ±3.01	272.48 ±3.72	260.01 ±4.98
Water 400	347.52 ±4.92	305.34 ±1.78	293.11 ±2.76	271.52 ±2.48	256.19 ±2.50
Chloroform200	347.46 ± 3.567	320.43 ±4.19	301.63 ±4.76	295.01 ±3.70	293.72 ±2.8
Chloroform400	342.82 ±3.62	318.20 ±4.45	305.30 ±3.34	298.38 ±2.60	302.41 ±3,62
Pet ether 200	350.72 ± 2.51	299.65 ±3.21	280.50 ±2.52	268.01 ±3.98	279.45 ±5.4
Pet ether 400	342.82 ±3.62	309.11 ±3.12	299.52 ±2.98	284.19 ±3.160	270.32 ±2.8
Standard	346.35 ±4.28	264.47 ± 3.16	258.90 ±2.51	247.46 ±2.77	212.67 ±2.36

Table 3: The comparative sub acute Antihyperglycemic effect of different Extracts on STZ induced Diabetic rats

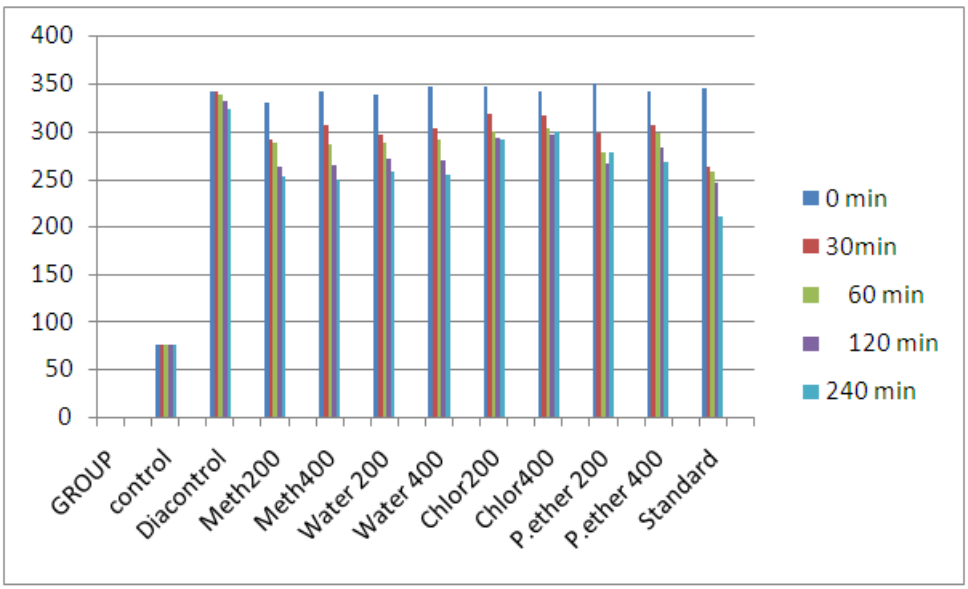
	Blood Glucose Level (mg/dl) at hr		
GROUP	**0 DAY**	**5TH DAY**	**15TH DAY**
control	75.75 ± 3.93	76.76 ± 3.5	75.80 ± 4.87
Diabetic control	343.37 ± 8.04	357.27 ± 7.54	380.56 ± 2.76
Methanol 200	332.67 ± 3.51	260 ± 4.8	145.46 ± 4.63
Methanol 400	342.32 ± 3.12	295.90 ± 7.28	209.32 ± 7.36
Water 200	338.30 ± 6.48	267 ± 6.3	152.27 ± 7.68
Water 400	342.32 ± 3.12	298.12 ± 5.19	208.52 ± 4.48
Chloroform200	336.36 ± 3.67	320 ± 3.54	290.48 ± 4.65
Chloroform400	332.75 ± 3.67	331 ± 3.34	272.90 ± 3.52
Pet ether 200	334.56 ± 4.90	290 ± 3.54	190.38 ± 3.28
Pet ether 400	345.53 ± 4.35	316 ± 4.78	210.32 ± 5.76
Standard	346.35 ± 4.28	226.53 ± 7.9	112.32 ± 46

Table 4: The comparative sub acute effect of different Extracts on body weight of rats

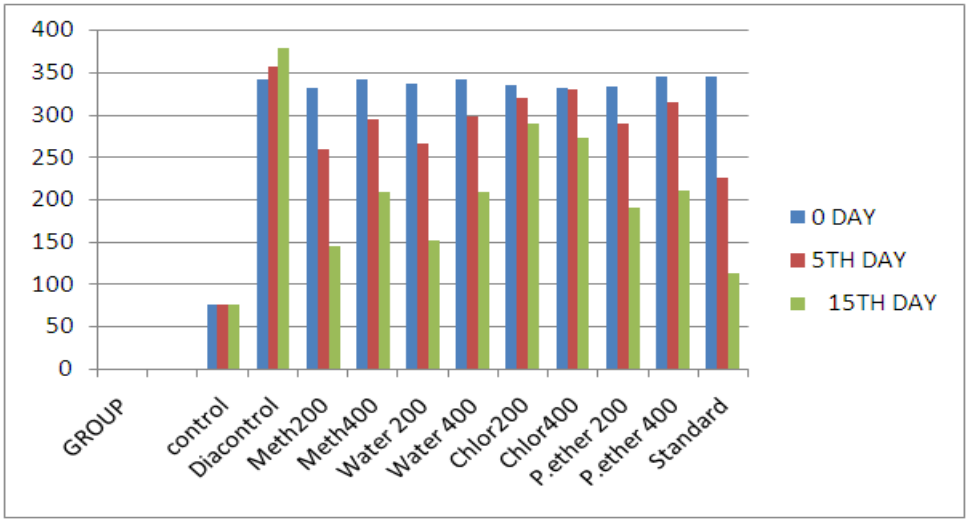
	Body weight in gram		
GROUP	**0 DAY**	**5TH DAY**	**15TH DAY**
Control	161.46± 3.21	162.37± 4.21	165.44± 4.55
Diabetic control	162.53± 3.54	158.40± 3.4	165.17± 5.4
Methanol 200	186.3± 4.78	192.2± 5.62	203.0± 3.97
Methanol 400	176.54± 5.5	154.2± 8.18	163.50 ± 3.8
Water 200	173.43 ± 3.94	177.35± 4.52	175.0±2.50
Water 400	175.25 ±4.25	173.42±6.21	174.35±3.4
Chloroform200	169.43+±3.16	165.45±3.53	162.78±4.64
Chloroform400	166.32±2.12	163.63±6.72	168.34±3.42
Pet ether 200	167.7± 5.13	163.65± 4.90	160.32± 6.32
Pet ether 400	169.34± 3.54	171.24± 4.89	164.42± 4.51
Standard	168.35±4.83	173.92± 3.79	178.40± 5.5



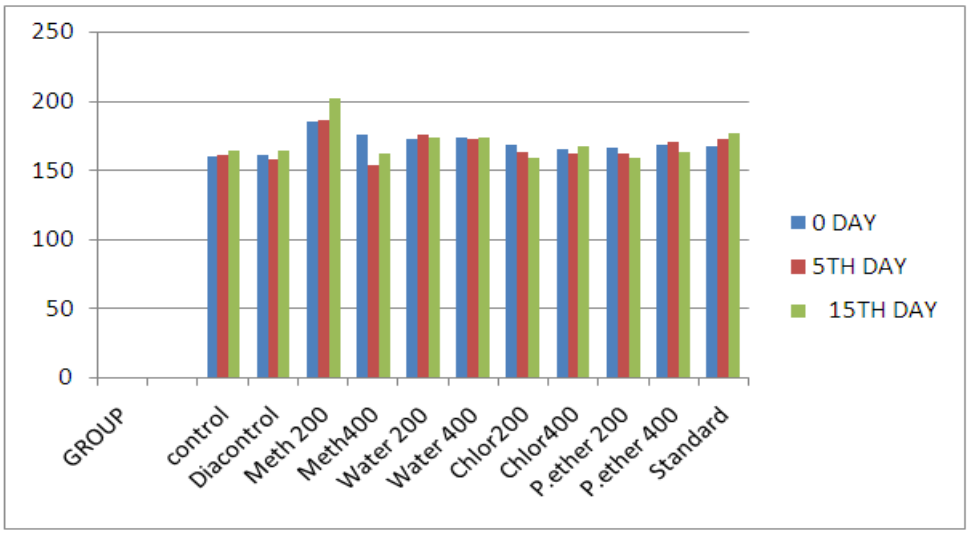
Graph 1: Anti diabetic effects of different extract of clitorea ternetea leaves on glucose loaded rat



Graph 2: The comparative Antihyperglycemic effect of different Extracts on STZ induced Diabetic rats



Graph3: The comparative sub acute Antihyperglycemic effect of different Extracts on STZ induced Diabetic rats



Graph 4: The comparative sub acute effect of different Extracts on body weight of rats

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