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RESEARCH ARTICLE

EFFECT OF CONCOMITANT ADMINISTRATION OF AQUEOUS EXTRACT OF LEAVES OF TRICHOSANTHES DIOICA AND METFORMIN IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

Background: In Ayurevdic practice T.dioica has been used to treat diabetes mellitus, alopecia and skin disorders. Hypoglycemic activity of leaf extract in normal and diabetic rats has been reported. But there is no report on its interaction with known drugs that are used in diabetes mellitus.

Aim: To study the effect of concomitant administration of aqueous extract of leaves of T.dioica and metformin on blood glucose in Streptozotocin induced diabetic rats.

Materials and methods: The aqueous extract of T.dioica (800mg/kg/p.o) alone and with metformin (250 and 500 mg/kg/p.o) were studied for their effect on fasting blood sugar in streptozotocin induced (45mg/kg/ip) diabetic rats.

Results: The administration of combination of T.dioica and metformin was significantly ($p<0.05$) more effective compared to either T.dioica or metformin alone.

Conclusion: T.dioica has enhanced the antihyperglycemic effect of metformin which may exploited for better management of diabetic patients

INTRODUCTION

Diabetes mellitus is a heterogeneous group of diseases, characterized by a state of chronic hyperglycemia, resulting from a diversity of etiologies, environmental and genetic, acting jointly.[1] Currently the number of cases of diabetes worldwide is estimated to be around 150 million. This number is predicted to double by 2025 with the greatest number of cases being expected in China and India.[2] Further it may be mentioned that in developing countries like India, majority of diabetic patients acquire the condition during most productive period of their lives, which is 45-64 years. Thus diabetes mellitus has now emerged as an important public health problem in India.

It is fairly easy to control the symptoms of diabetes with insulin, but it is difficult to maintain a normal blood sugar throughout 24 hours, even with the use of multiple injections of regular insulin or infusion pumps. Diabetes is usually irreversible and so requires treatment on a long term basis. The available treatment usually provides a good diabetic control, however it has its pitfalls. As an alternative mode of treatment, Ayurvedic medicine has been claimed to be less toxic and more efficacious.[3] In accordance with the recommendations of WHO expert committee on diabetes mellitus, an investigation of anti-hyperglycemic agents of plant origin used in traditional medicine seems important.[3] Many herbs and plant products have shown to possess anti hyperglycemic effect.[4, 5, 6, 7, 8]

In indigenous system of medicine Trichosanthes dioica (T. dioica, parwal or pointed gourd) is used to treat epilepsy, alopecia, skin disease and diabetes mellitus.[9] The fruits and seeds of the plant have been reported to have anti hyperglycemic activity.[10,11, 12] The leaves of the plant are used in diabetes mellitus but there is no scientific studies reported regarding the concomitant effect of T.dioica and metformin on diabetic rats, hence we planned to investigate the interaction between T.dioica and metformin in diabetic rats.

Material and Methods

Wistar rats (male) weighing between 200-250 g, raised in the Central Animal House of Manipal University, have been used in the study. They were fed on standard chow and given tap water ad libitum. The study was undertaken after getting the approval from the Institutional Animal Ethics Committee. We followed the guidelines set by the Indian National Science Academy, New Delhi, India for animal care and handling. A total of 36 rats were segregated into 6 groups of six animals each.

Preparation of plant extract

The dried leaves of T.dioica were crushed into moderately coarse powder. A kilogram of the coarse powder was immersed in distilled water in a flask for seven days. By straining the liquid the solid residue was obtained which was pressed and filtered. The filtrate was concentrated on water bath to get a viscous paste.[13] The viscous paste was dried in a desiccator and the extract(yield=4.25%) was given as a suspension in gum acacia.

Induction of Diabetes Mellitus

Preparation of 0.1M-citrate buffer solution was done by accurately weighing 1.49gm of trisodium citrate. It was dissolved in 100ml of cold distilled water and the necessary pH (4.5) was adjusted with concentrated HCL. A solution of streptozotocin (STZ) was prepared by dissolving the weighed quantity of STZ in0.1M freshly prepared ice-cold citrate buffer (pH 4.5) solution. The solution of STZ so prepared was administered in the volume of 0.5mL/200g.The selected rats were fasted overnight. Fasting blood glucose was measured and the rats were weighed before administering STZ 45mg/kg intraperitoneally.[14]After the procedure rats were placed in individual cage. Fasting blood glucose levels were determined on 6th day and 8th day and then on 12th day after administering STZ to confirm stable hyperglycemia.

Determination of efficacy of T.dioica and metformin in diabetic rats

The diabetic rats were divided into six groups of six rats each. Group 1- received vehicle, gum acacia (10ml/kg/p.o) (control). Group 2 received T.dioica (800mg/kg/p.o), group 3 and 4 received metformin at a dose of 250 and 500 mg/kg/p.o respectively. Group 5 and 6 received T.dioica (800mg/kg) + metformin (250mg/kg) and T.dioica (800mg/kg) + metformin (500mg/kg) respectively. The treatment was continued for 15 days. Fasting blood samples were collected at 0, 2, 4, 6, 12, 18, 24hrs after single dosing of rats for acute study. The sub-acute study involved repeated administration of T.dioica alone, metformin alone and their concomitant administration for 14 days(once daily) at predetermined time and blood glucose level were determined in samples withdrawn after 6hr of T.dioica alone, metformin alone and their concomitant administration on 0, 7th, 14thday. Blood glucose was measured even on 21st and 30th day to determine the duration of action of drugs (but the dosing was stopped on 14th day). Dose of T.dioica (800mg/kg) was decided based on the acute toxicity studies done by a previous research.[12]Two different metformin doses (250mg/kg and 500mg/kg) were decided based on a previous study.[15] The rats had free access to feed and water during the study period. Mean blood glucose and SEM were calculated.

Statistical analysis

Results are expressed as mean ±SEM. The data was analysed by one way ANOVA by Scheffe's post hoc test using SPSS computer package. $p<0.05$ was taken as the level of significance.

Results

Administration of T.dioica (800mg/kg) as well as metformin (250 and 500mg/kg) in single dose reduced blood glucose level at 2, 4 and 6hr after administration. The peak antihyperglycemic effect of T.dioica and metformin alone was observed at 2hr. The antihyperglycemic effect was found to reduce gradually at 4 hr and 6hr but waned at 24 hr. The peak anti-hyperglycemic effect in concomitant administration of T.dioica (800mg/kg) with metformin (250 and 500mg/kg) was at 2hrs and the duration of action was 12hrs and waned at 24hr. Concomitant administration of T.dioica with metformin 250mg/kg (group 5) showed significant ($p<.05$) reduction in blood glucose compared to T.dioica or metformin 250mg/kg alone at 4, 6, 12, 18hr. Concomitant administration of T.dioica with metformin 500mg/kg (group 6) showed significant ($p<0.05$) reduction in blood glucose compared to T.dioica or metformin 500mg/kg alone at 4, 6, 12, 18hr(Table 1)

In sub-acute study, T.dioica (800mg/kg) as well as metformin (250 and 500mg/kg) individually reduced blood glucose level significantly ($p<0.05$) on 7th, 14th, 21st day after administration. Repeated (once a day for 14 days) concomitant administration of T.dioica and metformin (250mg/kg) caused significant ($p<0.05$) reduction in blood glucose level as compared to T.dioica or metformin (250mg/kg) alone on 7th, 14th, 21st day. Repeated (once a day for 14 days) concomitant administration of T.dioica and metformin (500mg/kg) caused significant ($p<0.05$) reduction in blood glucose level as compared to T.dioica or metformin (500mg/kg) alone on 7th ,14th, 21st day. The values returned to base line by 30th day in all groups (Table 2).

Table 1: Effect of aqueous extract of Trichosanthes dioica (Td) alone, metformin alone and their concomitant administration on blood glucose level in diabetic rats (Acute study)

Sl.No	Groups	Blood glucose level in diabetic rats (mg/dl)						
		0 hr	2 hr	4 hr	6 hr	12 hr	18 hr	24 hr
1	Control	291±6.5	293±5.30	293±5.34	292±4.05	295±2.01	290±1.64	290±6.0
2	Td	287±6.3	263±17.4	269±15.1	272±10.8	280±10.2	285±3.49	284±6.5
3	M250	279±3.6	221±2.10	224±2.26	235±2.18	279±3.75	283±2.27	285±1.9
4	M500	284±3.3	198±1.98	204±2.97	215±2.67	274±5.44	286±2.64	278±143
5	Td+ M250	280±6.7	145±2.71[a,b]	157±1.60[a,b]	173±2.95[a,b]	181±4.64[a,b]	234±8.93[a,b]	277±3.4
6	Td+ M500	268±4.7	120±2.99[a,c]	131±3.52[a,c]	147±2.66[a,c]	159±2.90[a,c]	212±5.22[a,c]	277±2.7

n=6, values expressed as mean ±SEM
a = p< 0.05significant as compared to Td
b= p< 0.05 significant as compared to metformin 250mg/kg
c= p< 0.05 significant as compared to metformin 500mg/kg
M250=metformin 250mg/kg
M500=metformin 500mg/kg
Td= Trichosanthesdioica800mg/kg

Table 2: Effect of aqueous extract ofTrichosanthes dioica (Td) alone, metformin alone and their concomitant administration on blood glucose level in diabetic rats (Sub acute study)

Sl.No	Groups	Blood glucose level in diabetic rats (mg/dl)				
		0 day	7th day	14th day	21st day	30th day
1	Control	286±3.87	292±4.57	292±4.20	295±2.57	295±2.12
2	Td	287±3.28	192±2.51	157±3.16	252±8.53	293±2.31
3	M250	283±2.99	242±2.81	215±5.14	281±4.78	283±3.09
4	M500	289±5.33	220±2.79	186±4.06	287±3.17	294±2.43
5	Td+ M250	281±5.45	169±6.57[a,b]	131±4.06[a,b]	216±5.30[a,b]	284±4.60
6	Td+M500	290±3.94	140±2.19[a,c]	99±3.08[a,c]	209±5.69[a,c]	290±3.69

n=6, values expressed as mean ±SEM
a = p< 0.05significant as compared to Td
b= p< 0.05 significant as compared to metformin 250mg/kg
c= p< 0.05 significant as compared to metformin 500mg/kg
M250=metformin 250mg/kg
M500=metformin 500mg/kg
Td= Trichosanthes dioica 800mg/kg

Discussion

It has been shown that aqueous extract of the leaves of T. dioica has antidiabetic activity as it lowers serum glucose levels in diabetic rats. It also increases body weight of diabetic rats.[12] Theantidiabetic activity of T.dioica alone was limited to 6hr (with peak effect at 2hr). But concomitant administration of Td and metformin showed a sustained antihyperglycemic effect for 12hr, suggesting the advantage of concomitant administration in long term treatment. The phytochemicals present in T.dioica might have contributed to the synergistic antihyperglycemic effect with metformin which may be due to increased insulin secretion or/and increased glucose threshold. Repeated (once a

day for 14 days) concomitant administration of T.dioica and metformin caused significant ($p<0.05$) synergistic reduction in serum glucose level as compared to T.dioica or metformin alone. This shows it has a proven benefit when administered over prolonged period of time.

Induction of diabetes with streptozotocin is associated with a characteristic loss of body weight, which is probably due to muscle wasting.[16]In our study there was a significant weight loss in the control group, whereas rats which received treatment with T.dioica and concomitant treatment with T.dioica and metformin showed a lesser degree of weight loss compared to the control group. This indicates it has beneficial effect in preventing loss of body weight of diabetic rats. The probable mechanism of this benefit is due to its effect in controlling muscle wasting.[17]

As interest in the potential benefit of herbs and supplements for diabetes grows, it is very important to monitor the progress of the clinical literature and to communicate these findings to patients. Until more definitive studies help to clarify our questions, clinicians should remain cautious, yet open-minded, regarding adjunctive use of these supplements. They should be guided not only by sound clinical judgment, but also by patient's preferences, needs, and values. As we further our understanding of herbs and dietary supplements, we should begin to develop a framework for a medical system capable of incorporating those complementary therapies proven to be beneficial. Further studies are necessary to substantiate the above observation and to find out the active principle and exact mechanism of action involved in the antidiabetic activity of this plant.

Conclusion

The T.dioica plus metformin interaction observed in diabetic rats if extrapolated and confirmed in human diabetes would open a new avenue in the treatment of diabetes. T.dioica could be used as a useful adjuvant to achieve a better glycemic control, to prolong the action of metformin, to prevent the loss of body weight and also to increase the glucose tolerance in diabetic patients who are on metformin.

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