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EFFECT OF TETRAHYDROCURCUMIN IN STREPTOZOTOCIN - NICOTINAMIDE INDUCED DIABETES

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ABSTRACT

Diabetes is a major health problem affecting major populations worldwide. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro and micro-vascular dysfunctions. Pancreatic cell dysfunction and insulin resistance are the two hallmarks of type 2 diabetes mellitus. Treatment of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs are having so many side effects. Streptozotocin (STZ)-nicotinamide type 2 model shares a number of features with human type 2 diabetes and is characterized by moderate stable hyperglycemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion, in vivo and in vitro. Tetrahydrocurcumin (THC) is one of the major colorless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin. Curcumin is rapidly metabolized during absorption from the intestine, yielding THC, which has shown the strongest antioxidant activity among all curcuminoids. THC one of the active metabolites in curcumin on blood glucose and plasma insulin in streptozotocin induced diabetic rats. Different doses of THC (20, 40 and 80 mg\kg body weight) were orally administered to diabetic rats for 45 days, after which activities 6-weeks treatment with various doses of THC and curcumin on glucose levels were assayed.

Keywords: blood glucose, plasma insulin, pancreas, tetrahydrocurcumin, curcumin,

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (WHO, 2006). Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the five leading causes of death in the world (Ugochukwu and Babady, 2003; Devendra *et al.,* 2004). A recent study predicting the worldwide prevalence of diabetes will increase from 2.8% in 2000 to 4.4% in 2030, resulting in 366 million affected people (Wild *et al.,* 2004).

Pancreatic β-cell dysfunction and insulin resistance are the two hallmarks of type 2 diabetes mellitus. Treatment of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs are having so many side effects. Streptozotocin (STZ)-nicotinamide model shares a number of features with human type 2 diabetes. Hence, STZ-nicotinamide induced diabetes model was used in the present study (Murugan *et al.,* 2008).

Curcuma longa is commonly used in the treatment of diabetes by ayurvedic physicians. Curcumin is a biologically active component isolated from the rhizome of *Curcuma longa* that possess antihyperglycemic activity (Arun and Nalini, 2002), hypolipidemic action (Suresh Babu and Srinivasan, 1997) and anti - renal lesion effect (Suresh Babu and Srinivasan, 1998). The use of curcumin is recommended for prevention of advanced glycated end products (AGE) accumulation and the associated complications of diabetes (Sajithlal *et al.,* 1998).

Tetrahydrocurcumin (THC) is one of the major colourless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin (Majeed *et al.,* 1995 and Sugiyama *et al.,* 1996). Curcumin is rapidly metabolized during absorption from the intestine, yielding THC (Ravindranath and Chandrasekara, 1980), which has shown the strongest antioxidant activity among all curcuminoids (Osawa *et al.,* 1995). Several studies in experimental animals indicated that THC also prevent(s) cancer (Lin and Lin-Shiau, 2001), protect(s) against inflammation (Nakamura, 1998 and Hong *et al.,* 2004), atherosclerotic lesions (Naito *et al.,* 2002) and hepatotoxicity (Pari and Murugan, 2004). In our previous study, we have demonstrated the antidiabetic effect of THC in streptozotocin (STZ) induced diabetic rats (Pari and Murugan, 2005).

MATERIALS AND METHODS

Animals

Adult male albino Wistar rats (8 weeks), weighing 180 to 200 g bred in the Central Animal House, Rajah Muthiah

Table 1. Effect of 6-weeks treatment with various doses of THC on glucose levels in normal and experimental rats

Groups	'0' day	48 h after STZ injection	I week (after treatment)	II week	III week	IV week	V week	VI week
Blood Glucose (mg/dl)								
Normal	84.74 ± 4.64	85.51 ± 5.87	82.86 ± 5.27	83.57 ± 3.73	83.80 ± 4.83	82.12 ± 4.45	83.38 ± 5.18	83.78 ± 4.94
Normal + THC (80 mg)	84.16 ± 5.78	82.95 ± 5.27	80.51 ± 4.34	78.18 ± 3.48	74.62 ± 4.30	72.37 ± 3.22	73.85 ± 4.59	72..23 ± 4.26
Diabetic control	79.44 ± 4.93	255.15 ± 15.06**	258.75 ± 21.77**	279.58 ± 17.78**	303.56 ± 17.92**	321.92 ± 23.46**	329.66 ± 18.74**	330.57 ± 27.81**
Diabetic+ THC (20 mg)	77.51 ± 4.93	241.38 ± 20.53**	217.42 ± 12.20* (9.12)	199.51 ± 10.11** (17.42)	178.31 ± 11.81** (25.79)	158.31 ± 7.11** (34.07)	130.50 ± 6.37** (45.52)	108.18 ± 6.55** (55.19)
Diabetic+ THC (40 mg)	79.21 ± 4.30	247.00 ± 13.22**	221.29 ± 10.74* (11.21)	196.43 ± 9.71** (20.37)	163.53 ± 7.80** (34.44)	141.33 ± 5.38** (42.45)	117.16 ± 7.01** (52.35)	99.02 ± 4.19** (60.28)
Diabetic+ THC (80 mg)	78.56 ± 3.23	255.60 ± 16.40**	213.41 ± 10.45** (16.24)	176.91 ± 6.15** (31.44)	114.08 ± 8.58** (55.96)	107.30 ± 6.01** (58.61)	92.53 ± 6.35** (63.52)	87.19 ± 5.40** (65.63)
Diabetic + Curcumin (80 mg)	76.21 ± 4.38	245.53 ± 13.78**	216.32 ± 7.35* (10.70)	191.66 ± 9.0** (21.98)	118.36 ± 4.0** (51.86)	110.71 ± 5.26** (54.92)	97.50 ± 5.10** (60.18)	88.89 ± 6.52** (63.42)

Values are given as mean ± S.D for 6 rats in each group. Values in parentheses indicated the percentage lowering of blood glucose in comparison to basal reading after streptozotocin (STZ) administration at 48 h. Diabetic control was compared with normal. Experimental groups were compared with corresponding values after streptozotocin injection (48 h). * - $p< 0.01$, ** - $p<0.001$.

Medical College, Annamalai University, were used. All animal experiments were approved by the ethical committee (Vide. No: 284, 2005), Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of 24 ± 2° C, humidity of 45 to 64%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Drugs and chemicals

THC was a gift provided by Sabinsa Corporation, USA. Curcumin was purchased from Sigma chemicals company, St Louis, USA. All other chemicals and biochemical were of analytical grade.

Induction of diabetes

Non-Insulin dependent diabetes mellitus was induced (Masiello *et al.,* 1998) in overnight fasted rats by a single intraperitonial injection (i.p) of 65 mg/kg body weight STZ, 15 min after the i.p administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl will be used for the study.

Experimental design

In the experiment, a total of 42 rats (30 diabetic surviving rats, 6 normal rats) were used. The rats were divided into four groups of six each, after the induction of STZ diabetes. The experimental period was 45 days. Group I: Normal rats. Group II: Normal rats given THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group III: Diabetic control rats. Group IV: Diabetic rats given THC (20 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group V: Diabetic rats given THC (40 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group VI: Diabetic rats given THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group VII: Diabetic rats given curcumin (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Arun and Nalini 2002).

At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin.

Analytical procedure

Measurement of blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using

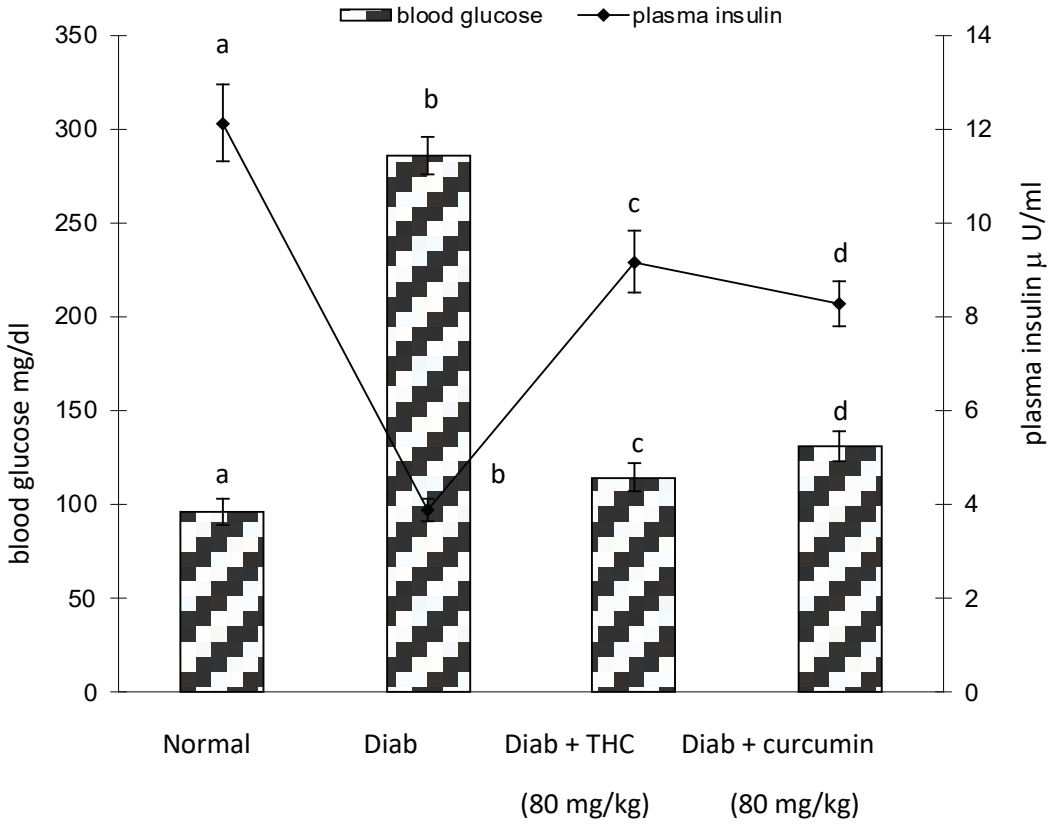

Figure 1. Effect of THC on the levels of blood glucose and plasma insulin in normal and experimental rats
Diab - Diabetes, THC - Tetrahydrocurcumin.
Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (John and Lott Turner, 1975). Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany).

Histopathological study

The pancreas samples fixed for 48h in 10% formal-saline were dehydrated by passing successfully in different mixture of ethyl alcohol – water, cleaned in xylene and embedded in paraffin. Sections of liver and kidney (4-5 ⊚m thick) were prepared and then stained with hematoxylin and eosin dye, which mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant if $p < 0.05$ (Duncan, 1957).

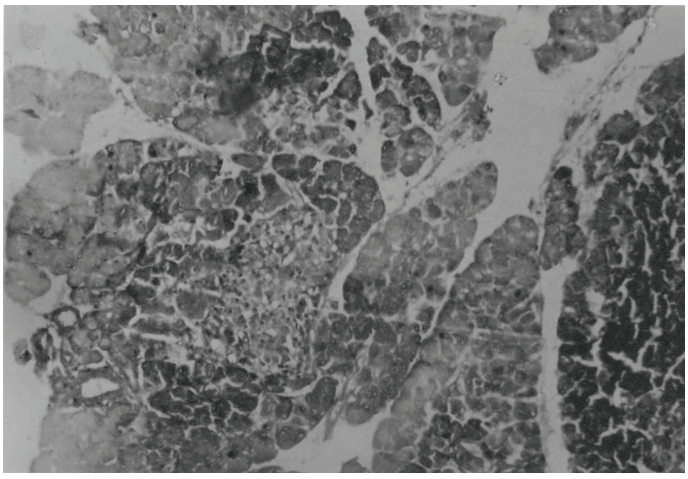

Figure 2A. Control rat pancreas. Normal pancrease with islets of langarhans cells

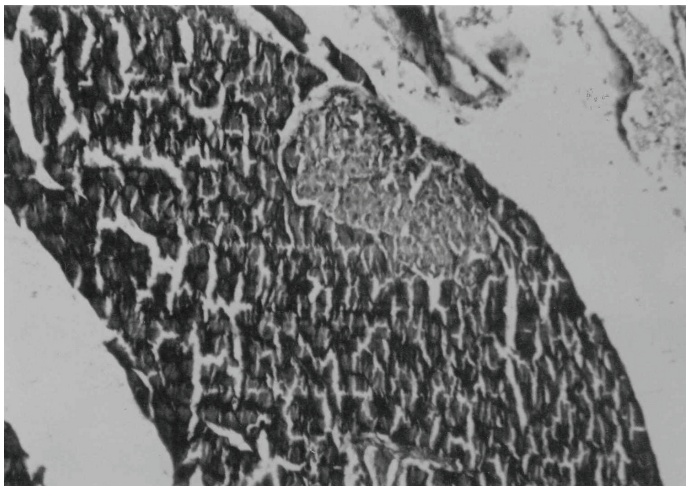

Figure 2B. Normal + THC treated rat pancreas. Normal architecture of pancreatic cells.

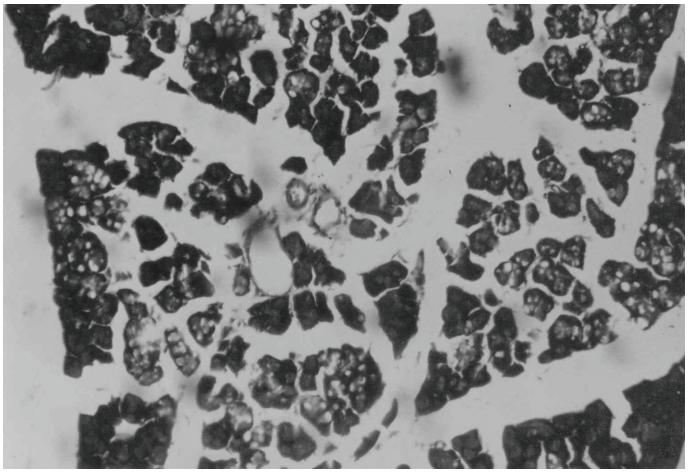

Figure 2C. Diabetic rat pancreas. Shows atrophic acini, no islet cells

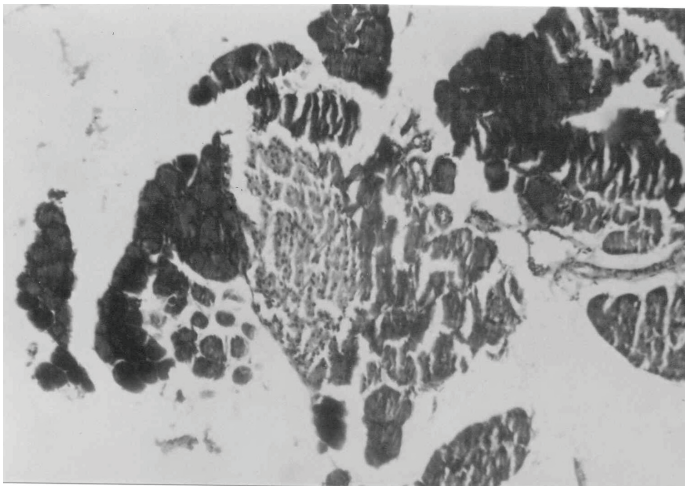

Figure 2D. Diabetic+ THC treated rat pancreas. Preservation of islet cells with few atrophic acini.

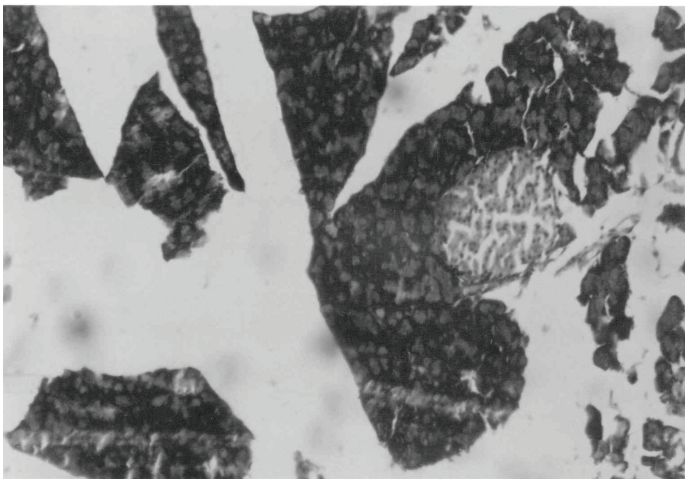

Figure 2E. Diabetic+ curcumin treated rat pancreas. Preservation of islet cells in the pancreas.

Figure 2. Histopathological changes in pancreas of control and experimental rats. All the sections are in H&E 100X.

RESULTS AND DISCUSSION

Effect of THC on blood glucose and plasma insulin

In all groups prior to streptozotocin administration, the basal levels of blood glucose of the rats were not significantly different. However, 48 h after streptozotocin-nicotinamide administration, blood glucose levels were significantly higher in rats selected for the study (Table 1). In contrast, non-diabetic controls remained persistently euglycaemic throughout the course of the study.

Table 1 shows the effect of treatment with THC and curcumin on blood glucose levels. In all the THC treated groups (20, 40 and 80 mg/kg), although a significant antihyperglycaemic ($p<0.01$) effect was evident from first week onwards, decrease in blood sugar was maximum on completion of the sixth week (65.63%) ($p<0.001$) in the group receiving 80 mg/kg/day of THC. On the basis of these studies, dose of 80 mg/kg per day of THC was selected for further evaluation. Administration of THC and curcumin to diabetic rats significantly increased the plasma insulin. The THC administration showed more effective than curcumin.

Changes in blood glucose and plasma insulin

Fig.1 shows the level of blood glucose and plasma insulin of different experimental groups. The diabetic control rats showed a significant increase in the level of blood glucose with significant decrease in the level of plasma insulin. Oral administration of THC to diabetic rats significantly reversed the above biochemical changes. In our previous study (Pari and Murugan, 2005) we have reported that THC at 80 mg/kg body weight showed better effect than 20 and 40 mg/kg body weight, therefore the 80 mg/kg body weight was used in this study. The administration of THC and curcumin to normal rats showed a significant effect on blood glucose and plasma insulin levels. The THC administration showed more effective than curcumin.

Histopathological observations

Pathological changes (Fig.2) of pancreas include shows atrophic acini, no islet cells in diabetic control rats (Fig. 2C). The above pathological changes were reduced in rats treated with THC and curcumin. C Diabetic control rat's pancreas showed Preservation of islet cells with few atrophic acini. These changes were reduced in THC and curcumin treated rats (Fig. 2D and 2E).

Discussion

DM is a chronic disorder of carbohydrates, fats and protein metabolism. A defective or deficient insulin secretary response, which translates into impaired carbohydrates (glucose) use, is a characteristic feature of diabetes mellitus, as is the resulting hyperglycemias (Kumar, 1992). DM is a non-communicable disease, which is considered as one of the five leading causes of death in the world (Ugochukwu and Babady, 2003; Devendra *et al*., 2004). The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Zimmet *et al*., 2001). The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (American Diabetes Association, 2002). The latest World Health Organization (WHO) estimate for

the number of people with diabetes, worldwide to be 370 million by 2030 (WHO, 2003).

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro and micro-vascular dysfunctions (Murugan and Pari, 2005).

Type 2 diabetes mellitus is a heterogeneous disease, characterized by low blood glucose control (intolerance to glucose) and result either from resistance to glucose in peripheral tissues (skeletal muscle and adipocytes) or relative decrease of ◎-cell activity. Depending on several factors (obesity, age and onset, severity of glucose intolerance and mode of inheritance), clinical features of individual suffering from type 2 diabetes are highly variable ((Murugan and Pari, 2007a).

In the present investigation, treatment with THC showed significant antihyperglycaemic activity. The maximum reduction in glucose levels was seen in groups receiving 80 mg/kg of the THC. This is probably indicative of efficacy of the plant. Moreover, it indirectly indicates that part of the antihyperglycaemic activity of this plant is due to release of insulin from the existing cells of pancreas. The possible mechanism of action of extract could be correlated with the reminiscent effect of the hypoglycaemic sulphonylureas which promote insulin secretion by closure of K+ - ATP channels, membrane depolarization and stimulation of Ca2+ influx, an initial key step in insulin secretion. In this context a number of other plants have also been reported to have antihyperglycaemic and insulin-release stimulatory effects (Murugan and Pari, 2007b).

In our study, histopathological observation in diabetic control rat's causes shows atrophic acini, no islet cells in the pancreas. The reaction is provoked by the increased production of highly reactive intermediates of STZ, which are normally detoxified by endogenous GSH but when present in excess, can deplete GSH stores, allowing the reactive intermediate to react with and destroy hepatic, renal cells (Blum and Fridovich, 1985). The above pathological changes were reduced in diabetic rats treated with THC and curcumin. The histological evidence of diabetic control rats suggest that structural alterations at the end of 45 days are due to STZ-induced free radical generation quite early in diabetes. Thus in addition to blood glucose lowering effect, histopathological observations also supports the notion that THC and curcumin at 80 mg/kg produced significant antihyperglycemic activity by protecting the tissues against STZ action. The protective effect of THC was more prominent compared with curcumin.

Administration of THC and curcumin has significant antidiabetic effect in STZ-nicotinamide induced diabetes. The THC and curcumin exhibited its antidiabetic effect by influencing the histopathological changes. The antidiabetic effect of THC provides sufficient documentation to define its role and action for its potential and promising use in treating diabetes. The THC administration showed more effective than curcumin.

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