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Hypoglycemic and hypolipidemic properties of hydroxychavicol, a major phenolic compound from the leaves of *Piper betle*linn. studied in high fat diet fed- low dose STZ induced experimental type 2 diabetes in rats

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ABSTRACT

Piper betle vine is bestowed with a unique placement in the list of traditionally important plants. Betel leaves are popularly known as "Green gold" because of their nutritive and medicinal values. Recently, we have reported the presence of Hydroxychavicol (HC),a phenolic secondary metabolite as a major constituent in the ethanolic extract of betel leaves.Diabetes mellitus is a major metabolic disorder characterized by dysregulation of carbohydrate, protein and lipid metabolism resulting from defects in insulin secretion, insulin action or both. Chronic hyperglycemia in diabetes leads to retinopathy, nephropathy, neuropathy and cardiovascular disease. Most of the currently available drugs for the treatment of diabetes often elicit undesirable side effects in addition to drug resistance after prolonged use. Search for novel drugs for the treatment of diabetes is still continuing due to the multifactorial nature of diabetes. Based on the folklore use, in the present study an attempt has been made to assess the hypoglycemic and hypolipidemic properties of hydroxychavicol, a major phenolic lead molecule present in the leaves of Piper betle L. in high fat diet fed - low dose STZ induced experimental type 2 diabetes in rats.Oral glucose tolerance test (OGTT) was performed to study the efficiency of hydroxychavicolon glucose homeostasis.Insulin resistance was also measured by HOMA-IR. The levels of fasting blood glucose, plasmainsulin, hemoglobin, glycosylatedhemoglobin, c-peptide and urine sugar were analyzed. Oral administration of hydroxychavicolat a concentration of 20mg/kg.b.w./rat/day to diabetic rats for a period of30dayssignificantlyimproved the altered levels of glucose homeostasis. Elevated levels of hepatic enzyme markers such as AST, ALT and ALP were reverted back to normal levels in diabetic group of rats with oral administration of hydroxychavicol. The altered activities of glycogen synthase and glycogen phosphorylase in hepatic tissues of diabetic rats were significantly reverted tonear normalcy upon oral treatment with hydroxychavicol. Additionally, the altered levels of lipid profile components observed in diabetic group of rats were normalized after oral treatment with hydroxychavicol. The efficacy of HC was comparable with metformin, a standard drug widely used for the treatment of diabetes. The results of the present study demonstrate the hypoglycemic and hypolipidemic properties of HC and also provide the scientific rationale for the use of Piper betle leaves in the treatment of diabetes.

Keywords: Diabetes mellitus, *Piper betle*, Hydroxychavicol, Antidiabetic,Antilipidemic,Metformin.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder arises from multiple biochemical and cellular defects characterized by absolute lack of insulin secretionfrom the pancreatic beta cells (T1DM) or its action in peripheral tissues (T2DM) [1]. More than 90% of the diabetic individuals belong to T2DM. Approximately 366 million people

are affected with diabetes in the year 2011, and this is expected to rise to 552 million globally by 2030, [2]making diabetes the most common cause of mortality after cancer. Around 80% of the people with diabetes are in developing countries, of which India and China share the larger proportions. This is a minimum number because, for each diagnosed case, there is thought to be one undiagnosed case in first world countries and eight in the third world countries[3]. The global increase in the prevalence of diabetes is due to population growth, increased aging population, urbanization, obesity and physical inactivity[4].Various non-pharmacological and pharmacological approaches are available to treat diabetes mellitus[5]. Most of the currently available drugs for the treatment of diabetes elicit undesirable side effects such as weight gain, gastrointestinal disorders and hypoglycemia. Additionally, these drugs develop resistance after prolonged use. Hence, search for new drugs with more efficacies and without side effect still continues. Plant extracts, as a natural blend of phytochemicals, offer immense opportunities for the discovery of active constituents, an archetype of current pharmaceutical industries[6]. However, only a few traditionally important medicinal plants have been subjected to pharmacological scrutiny. One such medicinal plant which lacks scientific evaluation for its traditional medicinal properties is *Piper betle*Linn.

Piper betle leaves are commonly referred to as "Green Gold". *Piper betle* Linn. (PBL) is the leaf of betel vine popularly known as "Paan" and "Betel" in English. It belongs to the dicotyledonous *Piperaceae* family[7]. It is a shade loving perennial root climber[8-10]. *Piper betle* vine is much more popular in India than in any other country of the World since the antiquity which is evident from the numerous citations laid down in the ancient literature, particularly in the Indian Scriptures. The significance of betel leaves has been explained in relation to every sphere of human life including social, cultural and religious and every day-to-day life which is very much relevant even these days [11]. The leaves along with sugar and areca nut is used as a special item offered to the guests in order to mark respect and such traditional use in the Indian society, the leaves nearly stand alone without any parallel even today[12]. The leaves mature about 15-20 days, 1 to 4 harvestings are normally performed every month and this may continue for 15-20 years or more [13]. About 15-20 million people consume betel leaves in India on regular basis besides those in other countries of the World may include over 2 billion consumers [11].

*Piper betle*vine is one of the popular plants which are integrated with cultural and traditional values in India. Irrespective of the traditional uses, betel vine is arguably the most maligned plant whose regular consumption is believed to cause cancer of the oral cavity. This infamous accreditation is principally due to the fact that habitual chewing of betel quid which consisting of areca nut, slaked lime, smokeless tobacco, in addition to betel leaves[14]. Several scientific reports have conclusively shown that the betel leaves are devoid of mutagenic as well as carcinogenic effects. Recent studies clearly evidenced that the phytochemicals present in the betel leaves significantly prevented chemically induced cancer in experimental animal models [15, 16].

Earlier, we have reported the antidiabetic activity of *Piper betle* leaves extract in alloxan-induced experimental diabetic rats[17] and more recently, we have isolated and characterized the biologically important phytochemicals such as Caffeic acid, p-Coumaric acid, Eugenol, Rutin and Hydroxychavicol from the leaves of *Piper betle*[18]. Since, hydroxychavicol was found to be present in relatively higher levels when compared to other phytoingredients, in the present study an attempt has been made to evaluate the antidiabetic and antilipidemic properties of hydroxychavicol in high fat diet fed - low dose STZ induced experimental diabetes in rats.

MATERIALS AND METHODS

Chemicals

Hydroxychavicol and STZ were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used in the study were of analytical grade and were obtained from standard commercial suppliers.

Experimental animals

Wistar Male albino rats, weighing about 160–180 g, were procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, India and housed in polypropylene cages lined with husk and maintained under standard experimental conditions. Prior to initiation of the experiments, the animals were acclimatized to standard husbandry conditions for a week to eliminate the effect of stress. The rats were fed with a commercial rat chow pellet (5% fat, 21% protein, 55% nitrogen free extract and 4% fibre [w/w]) with adequate vitamin levels for the animals (Hindustan Lever, Bangalore, India). All rats were provided with free access to water. The experimental protocols were conducted with the current ethical norms prescribed by the Ministry of Social Justices and

Empowerment, Government of India, and the approval of the Institutional Animal ethical committee (IAEC Approval No. : 04/01/2014).

Toxicity and dosage fixation studies

The acute toxicity of hydroxychavicol was studied in the control rats according to OECD guideline 423. Graded doses of hydroxychavicol dissolved in DMSO and were given orally and the animals were observed continuously for the first 2 hours followed by every hour up to 6 hours and daily thereafter for 14 days for any signs of morbidity, mortality and behavioral toxicity. Hydroxychavicol were found to be non-toxic upto 100 mg/kg b.w. Graded doses of hydroxychavicol(10, 20, 30, 40 mg/kg b.w) was administered to HFD + STZ induced diabetic rats for various periods of treatment. From the data obtained, the optimum dosage for the treatment of diabetes was fixed as 20 mg/kg b.w for 30 days.

Induction of diabetes and Experimental design

The rats were divided into four groups each comprising of not less than six animals. The rats were allocated into two dietary regimens by feeding either normal pellet diet (NPD) or high fat diet (HFD) for 2 weeks of dietary manipulation. The composition of HFD is powdered NPD – 365g/kg, Lard – 310 g/kg, Caseine – 250g/kg, cholesterol – 10g/kg, vitamin and mineral mix – 60g/kg, DL-methionine – 3g/kg, Yeast powder – 1g/kg, NaCl – 1g/kg.[19, 20].After 2 weeks of HFD maintenance the Group II-Group IV rats were injected with a single dose of STZ (35 mg/kg b.w. /rat), in 0.5ml of freshly prepared cold citrate buffer (pH 4.5) while the Group I rats fed with NPD were injected with citrate buffer in a same volume, intraperitoneally. After 6 h, and until 24h after STZ injection, STZ- injected rats were provided with 10% glucose solution to prevent diabetogen-induced hypoglycemia. After one week of STZ injection, rats with non-fasting blood glucose levels ≥ 250 mg/dl were chosen for further studies.

The rats were divided into four groups each comprising of a minimum of six rats as follows:
Group I: Control rats administered with vehicle alone.
Group II: HFD-STZ induced diabetic rats.
Group III: HFD-STZ induced diabetic rats orally treated with hydroxychavicol (20mg/kg b. w. /rat/day) for 30 days.
Group IV: HFD-STZ induced diabetic rats orally treated with Metformin (50 mg/kg b.w. /rat/day) for 30 days.

The rats were allowed to continue to feed on their respective diets until the end of the experiments. During the experimental period, body weight, blood glucose, food and water consumption and physical examinations were determined at regular intervals. At the end of 30 days of experimental period, the rats were fasted overnight, anesthetized with ketamine (80mg/kg, i.p.), and killed by cervical decapitation. Blood was collected with and without anticoagulants for plasma and serum separation, respectively. Liver tissues were excised from all four groups of rats, rinsed with ice-cold saline and stored at -70^{o}C until further use.

Oral glucose tolerance test (OGTT)

Overnight fasted rats of all groups were subjected to oral glucose tolerance test on the last week of the experimental period. The blood glucose levels were monitored at 0, 30, 60, 90 and 120 mins using One Touch glucometer (Life scan, Johnson and Johnson Company) after oral administration of 2 g/kg b.w. glucose as aqueous solution[21].

Assay on insulin resistance

The insulin resistance developed in the experimental animals was evaluated by a homeostasis model of insulin resistance (HOMA-IR). The HOMA-IR was calculated by the method of Mathews et al.(1985) [22] as follows

$$\text{HOMA-IR} = \frac{\text{Fasting insulin level (}\mu\text{U/ml) x Fasting blood glucose (mg/dl)}}{405}$$

Biochemical analysis

Fasting blood glucose, hemoglobin, glycosylated hemoglobin, plasma protein, blood urea, uric acid and serum creatinine levels were estimated [23-29]. Plasma insulin level was assayed using an ELISA kit (Linco Research, St Charles, MO, USA) for rat insulin assay. The presence of urine sugar was detected using urine strips (Diastix). The activities of pathological marker enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) in serum were assayed [30, 31].

A portion of the liver tissue was dissected out, washed immediately with ice-cold saline and was homogenized in 0.1M Tris–HCl buffer (pH 7.4) for the assay of key enzymes of glycogen metabolism. The supernatant obtained from the centrifugation of the liver homogenate was used as enzyme source for the assay of glycogen synthase and glycogen phosphorylase. Another portion of wet liver tissue was used for the estimation of glycogen content [32-34].

Assay of Lipid Profile

Serum levels of cholesterol, triglycerides (TGs)were estimated [35, 36]. HDLs and LDLs were separated from the serum according to a dual precipitation technique [37], and the cholesterol content of the lipoproteins was estimated.

$$\text{VLDL} = \frac{\text{Triglycerides (mg/dl)}}{5}$$

Statistical analysis

All the data were grouped and statistically evaluated with SPSS 16.0 software. Hypothesis testing methods included 'One-way analysis of variance' followed by 'least significant difference test' was used. A value of $P < 0.05$ was considered to indicate statistical significance. All results were expressed as mean ± Standard error mean (S.E.M) for six rats in each group.

RESULTS AND DISCUSSION

Recent reports on the etiology, epidemiology and consequences of T2DM necessitate the urgency to find better prognosis and prevention strategies. The currently available drugs for the treatment of T2DM have their own drawbacks ranging from development of resistance and adverse effects to lack of responsiveness in a large segment of patient population. Further, none of the glucose lowering agents adequately control hyperlipidemia that is frequently associated with T2DM [38]. Phytochemicals are non-nutrient secondary metabolites in plants which provide much of the colour and taste in fresh or processed food, fruits and vegetables [39]. Most of the phytochemicals such as polyphenols, glycosides, alkaloids, terpenoids, flavonoids, polysaccharides, tannins and saponins have been proposed for the treatment of various human ailments due to their significant antioxidant and related pharmacological properties [40, 41]. Among the phytoingredients, polyphenols play a pivotal role in photosynthesizing cells. Though, they are reported to possess significant antidiabetic properties, much is not studied about their specific mechanism of action [42]. In the present study, hydroxychavicol, a major phenolic compound present in the leaves of *Piper betle* vine was chosen to study its antidiabetic and antilipidemic properties.

Although, several animal models of natural as well as developed are available, the pattern of disease establishment and progression in most of them did not appear to be similar to the clinical status in humans[43, 44]. Recent studies have reported that the rats fed with high fat diet (HFD) and low dose of STZ administration could effectively be used to develop T2DM which closely resembles the natural history of the diseases in terms of insulin resistance and impairment of insulin secretion[45-47]. Hence, in the present study HFD fed -low dose STZ induced experimental T2DM was chosen as the animal model to evaluate the antidiabetic and antilipidemic properties of hydroxychavicol.

Figure 1 shows the effect of oral administration of hydroxychavicol on body weight in experimental group of rats. HFD-STZ induced diabetic rats showed reduction in body weight due to increased muscle wasting as well as loss of tissue proteins which is in agreement with previous reports [48, 49]. Loss of body weight denotes fat and protein catabolism due to altered glucose homeostasis, and polyuria indicates osmotic diuresis as a result of chronic hyperglycemia in HFD-STZ induced type 2 diabetic rats. Oral treatment with hydroxychavicol significantly improved the body weight gain. This could be the results from improved glycemic control elicited by hydroxychavicol.

Figure 2 shows the effect of hydroxychavicol treatment on oral glucose tolerance test in experimental group of rats. In control rats, the blood glucose level has reached the maximum peak at 60 mins after an oral glucose load and then it was gradually reverted back to near normalcy at 120mins indicating the existence normal glucose homeostasis. On the other side, the blood glucose levels in HFD-STZ induced diabetic rats reached the maximum peak at 60 mins and remained unsubsidized over the next 60 mins. Oral administration with hydroxychavicol as well as metformin resulted in a significant decrease in fasting blood glucose levels at 30 and 60 mins compared with diabetic group of rats. In addition, the blood glucose levels returned to basal level at 120 mins after the oral glucose load in

hydroxychavicol and metformin treated diabetic group of rats. OGTT is the only form of glucose tolerance test recommended for the diagnosis of insulin resistance in diabetes. The relationship between the levels of blood glucose and insulin after an external oral load of glucose can be studied using OGTT and is theoretically dependent on the rate at which carbohydrate enters the small intestine and glucose absorption as well as its insulin driven metabolism. OGTT is a measure of effective glucose utilization by the system and it is often performed for early diagnosis of diabetes [50]. It is considered as a more sensitive measure of early impairment in glucose homeostasis than fasting blood glucose or glycosylated hemoglobin levels. Thus, the results of the glucose tolerance test indicate the hypoglycemic effect of hydroxychavicol in maintaining glucose homeostasis.

HOMA-IR of control, diabetic, diabetic treated with hydroxychavicol and metformin groups of rats are depicted in Figure 3. Significant elevation in HOMA-IR values in diabetic group of rats indicating the development of insulin resistance. Diabetic rats exhibited significantly elevated fasting bloodglucose and HOMA-IR, accompanied by diminished plasma insulin levels. Therefore, it is suggested that insulin resistance developed in these rats exhibits chronic hyperglycemia which ultimately leads to the onset of T2DM.Oral treatment with hydroxychavicol, decreases the insulin resistance and improves impaired glucose tolerance, which manifest from the results of OGTT and HOMA-IR. It is also evidenced that hydroxychavicol acts as an insulin sensitizer likely due to glucose uptake in the main target organs. HOMA-IR is the biomarker that is often used to assess the extent of insulin resistance. Compared with the “gold” standard euglycemic clamp method for quantifying insulin resistance, quantification using HOMA-IR is more convenient[51].

The levels of fasting blood glucose, hemoglobin, glycosylated hemoglobin, C-peptide, plasma insulin and urine sugar of control and experimental groups of rats are shown in Table 1. The levels of fasting blood glucose were significantly increased in diabetic rats indicating the impairment of glucose metabolism due to abnormalities in the insulin secretion or its action. The observed decrease in the levels of hemoglobin and a concomitant increase in the levels of glycosylated hemoglobin in the experimental diabetic rats evidenced the existence of chronic hyperglycemic state. The significantly decreased levels of plasma insulin and c-peptide revealed the decrease in beta cell mass. Oral treatment with hydroxychavicol significantly improved in the alterations of above parameters indicating the insulin stimulatory effect of hydroxychavicol. Attenuation of hyperglycemic condition was achieved by hydroxychavicol treatment which may results from the improved insulin response. C-peptide is a cleavage product of insulin synthesis created in the pancreas as part of insulin production, and is released into the circulation with insulin[52].It interacts with cellular membranes at unidentified sites distinctive of the insulin family of receptors and signal to multiple targets known to play a crucial role in diabetes and diabetic complications, such Na^+ / K^+ ATPase and NOS[53].

The effect of hydroxychavicol on the levels of plasma protein, blood urea, serum uric acid and creatinine in experimental group of rats are presented in Table 2.The observed decrease in the levels of total protein with a concomitant increase in blood urea in diabetic group of rats may be attributed to increased muscle proteolysis and reduced protein synthesis. Diabetes mellitus is responsible for impairment of glucose metabolism, enhanced gluconeogenesis and decreased storage of proteins. Diabetes mellitus is usually accompanied by high urinary glucose concentration, which produces an osmotic diuresis and therefore polyuria. The most sensitive indicators of kidney injury include an increase in the levels of creatinine and urea in serum. Uric acid and creatinine can be used as a rough index of the glomerular filtration rate [54]. High levels of uric acid and creatinine indicates several disturbances in kidney [55]. Hydroxychavicol treatment significantly increased the levels of serum protein and decreased urea, uric acid and creatinine of diabetic rats. Diabetic rats treated with hydroxychavicolsignificantly inhibit proteolysis caused by insulin deficiency due to insulin resistance mainly in the glucose uptake cellsand improves total protein level to near normalcy and this mechanism of action of hydroxychavicol is comparable to that of metformin.

The activities of aspartate transaminase, alanine transaminase and alkaline phosphatase in the serum of control and experimental groups of rats are depicted in Table 3. The activities of ALT, AST and ALP were significantly elevated in HFD fed- low dose STZ induced diabetic group of rats.The increased activity of these cytoplasmic marker enzymes may be due to the cellular damage in the liver which is caused by STZ, the diabetogenic agent used for the induction of experimental diabetes [56, 57].Oral administration of hydroxychavicol to diabetic rats reverted back the activity of these enzymes to near normal indicating the tissue protective nature of the compound.

Table 4 represents the level of glycogen and the activities of glycogen synthase as well as glycogen phosphorylase in the liver tissues of control and experimental groups of rats. HFD fed-low dose STZ induced diabetic group of rats showed a significant decline in the glycogen level as well as in the glycogen synthase activity and a concomitant increase in the activity of glycogen phosphorylase. Oral treatment with hydroxychavicol as well as metformin to diabetic rats restored the level of glycogen and the activities of glycogen synthase as well as glycogen phosphorylase to near normalcy when compared to control group of rats. A main function of glycogen is to maintain a physiological blood glucose concentration, but only liver glycogen directly contributes to release of glucose into the blood.Net glycogen synthesis is determined by the balance of glycogen synthase and phosphorylase activities [58].In insulin resistant subjects, the fractional activity of glycogen synthase is low and correlates with the rates of glucose disposal[59].Breakdown of glycogen is limited by the activity of glycogenphosphorylase.

The effect of hydroxychavicol on the levels of total cholesterol, triglycerides and lipoproteins such as HDL, LDL and VLDL in experimental groups of rats were illustrated in Table 5 and 6.The levels of total cholesterol, triglycerides, LDL and VLDL were elevated significantly with concomitant decline in the levels of HDL in the diabetic group of rats than that of control group of rats. Further, the altered levels of these cholesterol, triglycerides and lipoproteins in the diabetic group of rats were significantly normalized by the oral administration of hydroxychavicol as well as metformin. Liver is the only organ that can catabolize and excrete quantitatively important amount of cholesterol[60].The typical lipid disorder in patients with diabetes, diabetic dyslipidemia, is characterized by elevated triglycerides, low levels of HDL cholesterol, and increased numbers of small, dense LDL particles. The strong association in increased LDL particles and elevated triglycerides appears to be linked to the altered insulin sensitivity common in the metabolic syndrome and type 2 diabetes. Insulin resistance promotes the conversion of energy from ingested carbohydrate into increased hepatic triglyceride synthesis, which in turn generates large numbers of atherogenic triglyceride rich lipoprotein particles, such as very low density lipoprotein (VLDL)[61].Phenolic compounds have been shown to have beneficial effects on dyslipidemia, which accelerates atherosclerosis in diabetes [62, 63]. Inactivation of hepatic AMPK is a key event in the pathogenesis of hypolipidemia in diabetes and polyphenols such as resveratrol and apigenin are reported to increase the phosphorylation of AMPK and its downstream target acetyl CoA- carboxylase and the increased activity of AMPK was found to be 200 times more potent than that of metformin, a synthetic polyphenol widely used for the treatment of diabetes mellitus[64].Our finding that structurally related dihydroxychavicol display comparable effects on hepatocellular lipids indicating that the activation of AMPK may account for its lipid lowering action.

Table 1: The levels of fasting blood glucose, glycosylated hemoglobin (HbA1c), plasma insulin and urine sugar in control and experimental groups of rats

Groups	Fasting Blood glucose	Hemoglobin	HbA1c	Insulin	C-Peptide	Urine sugar
Control	85.43 ± 4.47	13.88 ± 0.42	5.72 ± 0.20	15.82 ± 0.23	263.81 ± 1.64	Nil
Diabetic control	254.84 ± 1.98^{a*}	08.13 ± 0.35^{a*}	12.08 ± 0.28^{a*}	9.15 ± 0.41^{a*}	126.61 ± 0.99^{a*}	+++
Diabetic + Hydroxychavicol	133.04 ± 2.98^{b*}	13.09 ± 0.27^{b*}	6.35 ± 0.16^{b*}	11.35 ± 0.32^{b*}	212.24 ± 1.71^{b*}	Nil
Diabetic + Metformin	123.99 ± 5.69^{b*}	13.29 ± 0.16^{b*}	5.90 ± 0.20^{b*}	12.38 ± 0.44^{b*}	237.03 ± 1.55^{b*}	Nil

Units: mg/dl for blood glucose, % hemoglobin for HbA1c, µU/ml for plasma insulin, C-Peptide- (µU/ml),+++ indicates more than 2% sugar. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with [a]Control rats, [b]Diabetic rats. Values are statistically significant at * P<0.05

Table 2: Effect of Hydroxychavicolon the levels of plasma protein, blood urea, serum uric acid and serum creatinine in experimental groups of rats

Groups	Protein	Urea	Uric acid	Creatinine
Control	8.96 ± 0.32	19.16 ± 0.49	2.43 ± 0.09	0.58 ± 0.06
Diabetic control	6.12 ± 0.20^{a*}	48.15 ± 0.81^{a*}	5.63 ± 0.15^{a*}	1.69 ± 0.04^{a*}
Diabetic + Hydroxychavicol	7.85 ± 0.27^{b*}	23.01 ± 0.62^{b*}	2.79 ± 0.06^{b*}	0.69 ± 0.01^{b*}
Diabetic + Metformin	8.07 ± 0.29^{b*}	21.81 ± 0.71^{b*}	2.30 ± 0.04^{b*}	0.57 ± 0.02^{b*}

Units: g/dl for plasma protein, mg/dl for blood urea, serum uric acid and serum creatinine. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. Values are statistically significant at * P<0.05. The results were compared with [a]Control rats, [b]Diabetic rats

Table 3: Effect of Hydroxychavicolon the levels of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum of experimental groups of rats

Groups	Control	Diabetic control	Diabetic + Hydroxychavicol	Diabetic + Metformin
AST	80.84 ± 1.14	128..70 ± 2.61[a*]	92.21 ± 1.18[b*]	84.37 ± 2.22[b*]
ALT	20.21 ± 0.28	44.00 ± 0.80[a*]	24.81 ± 0.50[b*]	18.61 ± 0.75[b*]
ALP	75.15 ± 1.07	143.90 ± 1.10[a*]	90.37± 0.76[b*]	70.15 ± 1.51[b*]

Enzyme activities are expressed as: AST and ALT - µmoles of pyruvate liberated/h/mg of protein, ALP - µmoles of phenol liberated/min/mg of protein. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. Values are statistically significant at * P<0.05. The results were compared with [a]Control rats, [b]Diabetic rats

Table 4: Level of glycogen content and activities of glycogen synthase and glycogen phosphorylase in liver tissues of control and experimental groups of rats

Groups	Glycogen	Glycogen synthase	Glycogen phosphorylase
Control	55.78±1.40	851.22 ± 10.46	651.32± 11.82
Diabetic	29.34±0.17 [a*]	519.20 ± 15.52 [a*]	865.10 ± 12.51 [a*]
Diabetic + Hydroxychavicol	43.00±0.85 [b*]	746.11 ± 19.70[b*]	734.11 ± 15.08[b*]
Diabetic + metformin	49.23±0.75 [b*]	784.71 ± 12.25[b*]	732.51± 14.51[b*]

Units are expressed as: mg of glucose/g wet tissue for glycogen, µmoles of UDP formed/h/mg protein for glycogen synthase and µmoles Pi liberated/h/mg protein for glycogen phosphorylase.Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. The results were compared with [a]Control rats, [b]Diabetic rats Values are statistically significant at [*]P<0.05

Table 5: The levels of cholesterol and triglycerides of control and experimental rats after 30 days of experimental period

Groups	Cholesterol	Triglycerides
Control	115.72 ± 1.79	72.85 ± 2.04
Diabetic	235.03 ± 10.02[a*]	169.09 ± 16.07[a*]
Diabetic + Hydroxychavicol	152.28 ± 4.40[b*]	110.15± 4.46 [b*]
Diabetic + metformin	134.14 ± 2.66[b*]	111.49 ± 4.36 [b*]

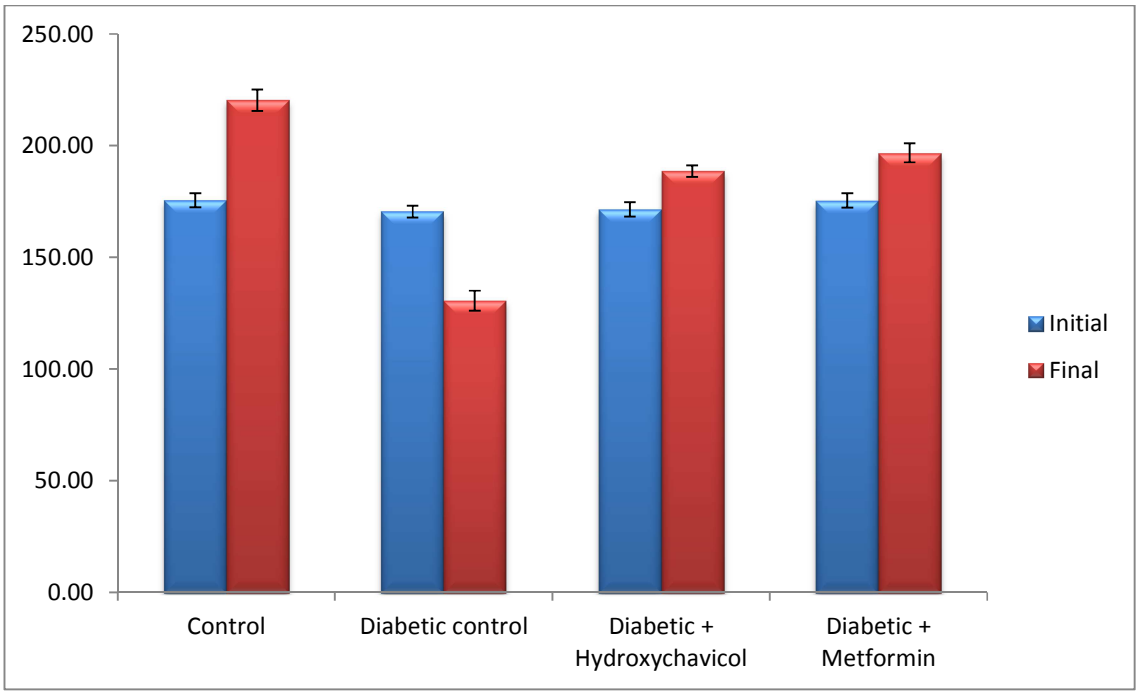
Units: mg/dl. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. The results were compared with [a]Control rats, [b]Diabetic rats. Values are statistically significant at *P<0.05

Table 6: The levels of lipoprotein cholesterol in control and experimental groups of rats after 30 days of experimental period

Groups	HDL	LDL	VLDL
Control	28.77 ± 1.63	52.06 ± 4.10	14.89 ± 0.45
Diabetic	10.85 ± 0.47 [a*]	179.88 ± 9.24 [a*]	33.46 ± 0.92 [a*]
Diabetic + Hydroxychavicol	21.52 ± 1.15[b*]	105.26 ± 3.80[b*]	20.40 ± 0.75[b*]
Diabetic + metformin	20.21 ± 1.05[b*]	97.62 ± 5.22[b*]	19.27 ± 0.92[b*]

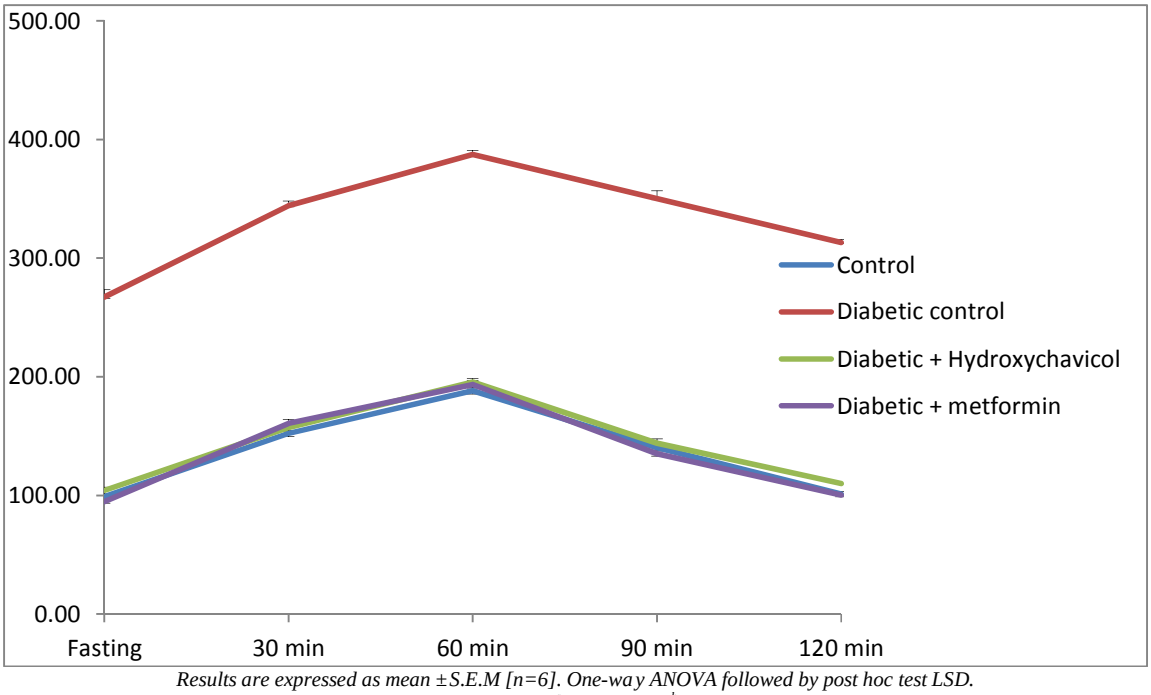
Units: mg/dl. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. The results were compared with [a]Control rats, [b]Diabetic rats. Values are statistically significant at *P<0.05

Figure 1: Effect of oral administration of hydroxychavicol on body weight in experimental group of rats



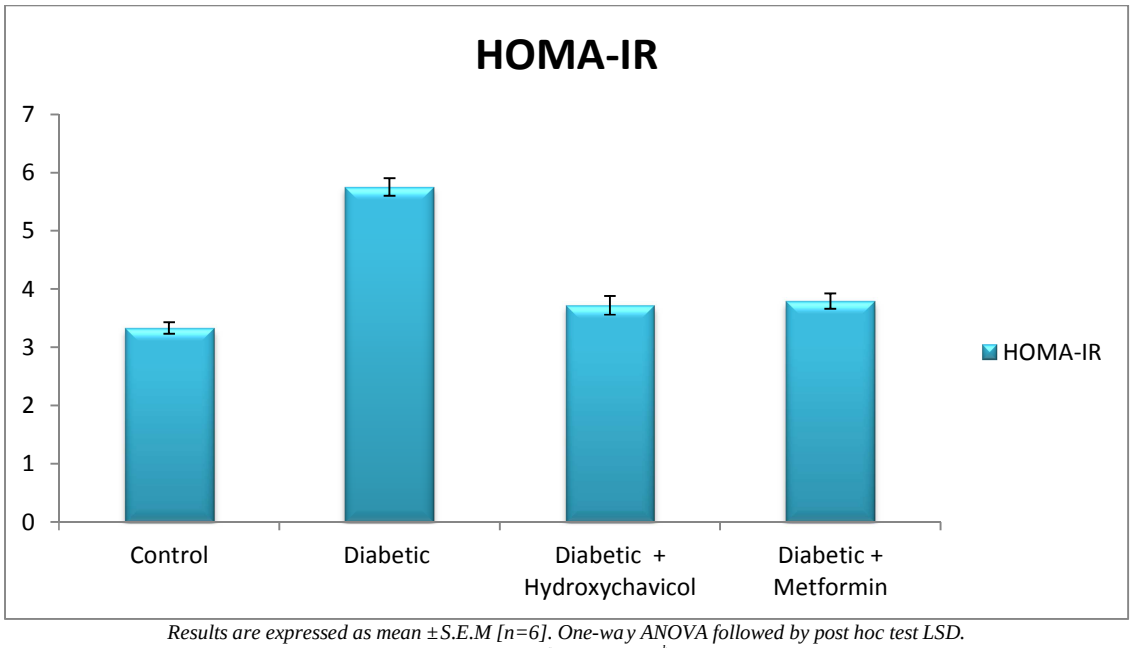
Results are expressed as mean ±S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD.
Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats. Values are statistically significant at [*]p<0.05

Figure 2: Effect of hydroxychavicol treatment on oral glucose tolerance test in experimental group of rats



Results are expressed as mean ±S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD.
Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats. Values are statistically significant at [*]p<0.05

Figure 3: HOMA-IR of control, diabetic, diabetic treated with hydroxychavicol and metformin groups of rats



Results are expressed as mean ±S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD.
Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats. Values are statistically significant at [*]p<0.05

CONCLUSION

The results of the present study evidenced that hydroxychavicol is an effective antidiabetic lead molecule as it possess the ability to enhance insulin secretion from the remnant beta cells of pancreas and to decrease hepatic glucose production along with increased insulin sensitivity. Additionally, it seems that hydroxychavicol is beneficial against hyperlipidemia, a major secondary complication of diabetes mellitus thereby delaying the development of diabetes and its major secondary complications. Since hydroxychavicol is non-toxic and found to possess significant hypoglycemic and hypolipidemic properties, it may be consider as a potential candidate for the treatment of type 2 diabetes mellitus.

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