Antidiabetic and antidyslipidemic nature of carvacrol, a monoterpenic phenol studied in high-fat -fed and low-dose streptozotocin-induced experimental diabetic rats

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ABSTRACT

Background: Carvacrol, a monoterpenic phenol found to be present in many essential oils of the family Labiatae including Origanum, Satureja, Thymbra, Thymus, and Corydothymus species. It exerts various beneficial and pharmacological properties. The present study was designed to investigate the antidiabetic properties of carvacrol in high fat fed - low dose streptozotocin-induced experimental type 2 diabetes in rats. Methods: Experimental type 2 diabetes was induced with high fat fed diet and low dose streptozotocin. Diabetic rats were treated with Carvacrol (20 mg/Kg b.wt/rat/day) for 30 days. The toxicological parameters such as AST, ALT and ALP were assayed. Biochemical parameters such as fasting blood glucose, glycosylated hemoglobin, insulin, insulin resistance and lipid profile were measured. The levels of glycogen in muscle and liver tissues were determined. Results and discussion: Diabetic rats were treated with Carvacrol (20 mg/Kg b.wt/rat/day) for 30 days. The toxicological parameters such as AST, ALT and ALP as well as biochemical parameters such as fasting blood glucose, glycosylated hemoglobin, insulin, insulin resistance and lipid profile were measured. Oral treatment with carvacrol significantly decreased the elevated levels of fasting glucose, glycosylated hemoglobin, AST, ALT and ALP. The insulin level was improved with an improvement in hepatic and muscle glycogen content of insulin resistant diabetic rats. Carvacrol also normalized the status of lipid profile. These results showed that carvacrol possess significant antihyperglycemic and antidyslipidemic effects in HFD/STZ-induced type 2 diabetic rats. Conclusion: The results showed that carvacrol exerts potential anti-hyperglycemic and antidyslipidemic effects in HFD/STZ-induced type 2 diabetic rats.

KEY WORDS: Carvacrol; High fat diet; Streptozotocin; antidiabetic; antidyslipidemic.

INTRODUCTION

Diabetes mellitus is a multifactorial and multisystemic metabolic disorder characterized by altered glucose and lipid metabolism resulting in persistent hyperglycaemia. The incidence of diabetes mellitus is increasing globally and is speculated that about 552 million people would be affected worldwide by the year 2030 [1]. Chronic hyperglycaemia in diabetes is accompanied by insulin resistance, which plays a key role in the initiation and progression of type 2 diabetes (T2D). Abnormal regulation of glucose and lipid metabolism and an increase in both fasting as well as postprandial glucose and lipid levels are the consequences of insulin resistance [2].

T1DM patients depend on externally administered insulin, while for T2DM patients who do not respond to diet and exercise regimes, oral anti-diabetes drugs (OADs) and sometimes external insulin are administered to maintain normoglycemia [3]. Though drugs are plenty for the treatment of diabetes, none is found to be effective due to undesirable side effects or diminution after prolonged use. Phytotherapy continue to play an important in the treatment of DM. Amelioration of T2DM risk usually targets lifestyle and diet, primarily with the aim of reducing obesity, the foremost risk factor in the development of insulin resistance and ultimately T2DM.

High fat diet induced insulin resistance model is the most widely used experimental type 2 diabetic animal model and is shown to increase hepatic glucose production and decrease insulin sensitivity which are the main characteristic features of type 2 diabetes [4]. Streptozotocin (STZ), a glucose analogue, is widely used to induce both type 1 and type 2 diabetes mellitus by inducing β cell death through alkylation of DNA. A high dose of STZ strongly impairs insulin secretion mimicking type 1 diabetes whereas a lowdose of STZ has been shown to
induce a mild impairment of insulin secretion which resembles the clinical features similar to type 2 diabetes \cite{5,6}. Hence, in the present study a combination of high fat diet and low dose STZ has been employed to induce experimental type 2 diabetes in rats. Even though antihyperglycemic drugs are necessary for the treatment of T2DM, oral hypoglycemic agents exhibit undesirable side effects. Thus, there is a necessity of oral antidiabetic agents without any adverse effect. A wide variety of phytochemicals, with minimal side effects, provide an alternative therapy for T2DM.

Carvacrol or cymophenol (2-methyl-5-isopropyl phenol), is a monoterpenic phenol which occurs in many essential oils of the family \textit{Labiatae} including Origanum, Satureja, Thymbra, Thymus, and Corydothymus species \cite{7} and also in piper betel leaves. However, the oil from \textit{origanum vulgare} has the appreciable amounts of carvacrol about 80% and is thought to be the most biologically active compound responsible for its medicinal value \cite{8}. It is well known that essential oils, which are rich in carvacrol, exerts antioxidant properties equivalent to those of ascorbic acid, butyl hydroxytoluene (BHT), and vitamin E \cite{9,10}. Carvacrol exerts pharmacological activities such as anti-inflammatory \cite{11}, antitumor \cite{12}, and antimicrobial \cite{13} and also it activates peroxisome proliferator-activated receptor and suppresses COX-2 inflammation \cite{14}.

In the absence of systemic studies in the literature, the present study was aimed to evaluate the antihyperglycemic and antihyperlipidemic potential of carvacrol in high fat fed low dose streptozotocin induced type 2 diabetic rats.

**Experimental Animals**

Male albino wistar rats weighing 150-170g were purchased and maintained in Ecbiology lab of Voorhees College, Vellore. The rats were housed in polypropylene cages lined with husk. They were maintained at an ambient temperature of 25 ± 2°C and 12/12 h of light/dark cycle. Animals were fed with standard commercial rat chow (Hindustan Lever Ltd) and water \textit{ad libitum} and housed under standard environmental conditions throughout the study. The experiments were strictly conducted according to the ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

**High fat diet fed streptozotocin induced diabetes**

The rats were divided into two dietary regimens by feeding either normal or high fat diet (HFD) for the initial period of two weeks \cite{15}. 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. After two weeks of dietary manipulation, the groups of rats fed with HFD was injected intraperitoneally (IP) with a low dose of STZ (35 mg/kg b.w) dissolved in 0.1M cold citrate buffer, pH 4.5). One week after STZ injection, the rats were screened for blood glucose level. Rats having fasting blood glucose (FBG) >250mg/dl that exhibited random hyperglycaemia and glycosuria were selected for the experiment. The rats were allowed to continue to feed on their respective diets until the end of the experiments.

**Toxicity and dosage fixation studies**

The acute toxicity of carvacrol was studied in the control rats according to OECD guideline 423. Different doses of carvacrol dissolved in water 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 fourteen days for any signs of morbidity, mortality and behavioral toxicity. Carvacrol was found to be non-toxic up to 2 g/kg b.w.

Graded doses of carvacrol (10, 20, 30, 50 mg/kg b.w) was administered to HFD + STZ induced diabetic rats for various periods of treatment. From the data obtained, the optimum dosage was fixed as 20 mg/kg b.w for 30 days. The animals were divided into four groups, comprising a minimum of six animals in each group as follows:

- **Group 1** – Control rats.
- **Group 2** – HFD+STZ (i.p. 35mg/kg b.w.) induced diabetic rats.
- **Group 3** – Carvacrol (20 mg/kg b.w.) treated diabetic rats
- **Group 4** – Diabetic rats treated with metformin (200 mg/ kg b.w/ day) in aqueous solution orally for 30 days.
At the end of the treatment period, the rats were fasted overnight, anesthetized and sacrificed by cervical decapitation. The blood was collected with and without anticoagulants for plasma and serum separation, respectively.

**Oral Glucose Tolerance Test**
On the day prior to sacrifice, Oral glucose tolerance test (OGTT) test was performed in all the groups. Blood samples were obtained from the lateral tail vein of rats deprived of food overnight. Successive blood sample was taken at 0, 30, 60, 90 and 120 minutes following the administration of 2 g/kg b.w. of glucose solution.

**Biochemical parameters**
Fasting blood glucose level was estimated according to the method of Sasaki et al., 1972 [16]. Plasma insulin was assayed using the Ultra-sensitive ELISA kit for rat insulin (Linco Research, St Charles, MO, USA). Glycosylated hemoglobin (HbA1c) levels was estimated according to the method of Nayak and Pattabiraman [17]. Urine sugar was detected using commercially available diastix urine strips. Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were assayed [18, 19]. The liver tissues were dissected out and washed with ice-cold saline for determination of glycogen content [20].

**Determination of homeostasis model of insulin assessment**
As the insulin abnormality cannot be accurately detected by a single determination of insulin or glucose levels, the insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR) as follows [21]

\[
\text{HOMA-IR} = \frac{\text{Fasting insulin level (µU/ml)}}{\text{Fasting blood glucose (mg/dl)}}/405
\]

**Lipid profile studies**
Cholesterol content [22], triglycerides [23] and free fatty acids in serum were estimated [24].

**Statistical analysis**
The results were expressed as mean ± S.E.M of six rats per group and statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS (version 16) program followed by LSD. Values were considered statistically significant when p < 0.05.

### 3. RESULTS
The levels of blood glucose in certain durations after the oral administration of glucose (2g/Kg body weight) in normal and experimental group of rats is depicted in table 1. In control rats, the blood glucose level reached the maximum peak at 60 min after an oral glucose load and then it was gradually reverted back to near normal levels after 120 min. In STZ-induced diabetic rats, the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. Oral administration of carvacrol to HFD-STZ induced diabetic rats showed significant decrease in blood glucose concentration at 60 and 120 min when compared with diabetic control.

The effect of carvacrol on the levels of fasting blood glucose, fasting plasma insulin and glycosylated hemoglobin (HbA1c%) in HFD/STZ diabetic rats is shown in Table 1. The levels of blood glucose and HbA1c% was found to be significantly elevated in diabetic rats as compared with normal control. Oral administration of carvacrol to diabetic rats significantly improved the altered level. The levels of plasma insulin were moderately decreased in HFD-STZ induced diabetic rats. Diabetic rats treated with carvacrol and metformin showed improved insulin level.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
<th>HbA1c (%)</th>
<th>Insulin (µU/ml)</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.91 ± 4.52</td>
<td>5.18 ± 0.22</td>
<td>15.04 ± 0.19</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic</td>
<td>263.11 ± 12.21</td>
<td>12.42 ± 0.35</td>
<td>10.29 ± 0.45</td>
<td>+++</td>
</tr>
<tr>
<td>Diabetic + Carvacrol</td>
<td>133.10 ± 5.05</td>
<td>7.05 ± 0.28</td>
<td>11.10 ± 0.32</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic + metformin</td>
<td>121.20 ± 6.16</td>
<td>6.62 ± 0.23</td>
<td>12.32 ± 0.43</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Units: mg/dl for blood glucose, % hemoglobin for HbA1c, µU/ml for plasma insulin, +++ indicates more than 2% sugar. 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 *Control rats; Diabetic rats.*

**Fig 1.** The effect of oral administration of carvacrol on levels of blood glucose in experimental groups of rats after receiving an oral glucose load.
Liver and muscle glycogen content of HFD/STZ diabetic control rats showed a highly significant decrease as compared to normal control (Figure 2). However, treatment with carvacrol and metformin altered the levels of glycogen content.

Table 2 represents the effect of carvacrol on the activities of serum AST, ALT and ALP in the experimental groups of rats. The pathophysiological indices in diabetic group of rats were significantly (p < 0.05) elevated as compared with control group of rats. Oral administration of carvacrol to diabetic groups of rats significantly (p < 0.05) normalized the altered levels in comparison with control group of rats. The results are comparable with the standard drug metformin.

The effect of carvacrol on lipid profile of diabetic rats are presented in Figure 4. Diabetic rats exhibited a highly significant increase in serum cholesterol, triglycerides and FFAs as compared with the control group. The administration of carvacrol led to marked amelioration of all parameters of the altered lipid profile.

**Table 2.** The activities of serum transaminases and alkaline phosphatase in the control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.53 ± 1.34</td>
<td>21.90 ± 0.39</td>
<td>72.64 ± 0.83</td>
</tr>
<tr>
<td>Diabetic</td>
<td>131.75 ± 2.31a*</td>
<td>43.81 ± 1.57b*</td>
<td>143.14 ± 7.13a*</td>
</tr>
<tr>
<td>Diabetic + Carvacrol</td>
<td>82.50 ± 2.45a*</td>
<td>24.17 ± 0.75b*</td>
<td>93.11 ± 1.36a*</td>
</tr>
<tr>
<td>Diabetic + Metformin</td>
<td>72.17 ± 1.49a*</td>
<td>18.72 ± 0.69b*</td>
<td>73.88 ± 1.57a*</td>
</tr>
</tbody>
</table>

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. The results were compared with *Control rats, bDiabetic rats. Values are statistically significant at *P<0.05.

The effect of carvacrol on insulin sensitivity in experimental groups of rats.

Fig 2. The effect of oral administration of carvacrol on insulin sensitivity in experimental groups of rats.

Values are given as mean ± S.E.M (n=6) one way ANOVA followed by post hoc test LSD. *p<0.05, when compared with aControl rats; bDiabetic rats

HOMA-IR of normal, diabetic and diabetic treated with carvacrol is depicted in Figure 3. Diabetic rats showed a significant elevation of HOMA-IR and it was decreased significantly upon the administration of carvacrol.

Fig 3. The effect of carvacrol on the liver and muscle glycogen content in the experimental groups of rats.

Values are given as mean ± S.E. M (n=6) one way ANOVA followed by post hoc test LSD. *p<0.05, when compared with aControl rats; bDiabetic rats

The effect of carvacrol on the levels of cholesterol, triglycerides and free fatty acids in the experimental groups of rats.

Fig 4. The effect of carvacrol on the levels of cholesterol, triglycerides and free fatty acids in the experimental groups of rats.

Values are given as mean ± S.E. M. (n=6) one way ANOVA followed by post hoc test LSD. *p<0.05, when compared with aControl rats; bDiabetic rats
DISCUSSION
The development of an ideal model for type 2 diabetes that would closely reflect the natural history and metabolic characteristics of human T2DM is very challenging. High fat diet mediates insulin resistance through reduced tyrosine phosphorylation of insulin receptor substrate (IRS), a crucial receptor which facilitates the signals that stimulate insulin action. Furthermore, low dose STZ prompts mild impairment to pancreatic β-cells which is parallel to the metabolic characteristics of the later stage of T2DM.

The present study revealed that the HFD-STZ induced type diabetic rats showed a significant increase in glucose level. This elevation in plasma glucose levels in diabetic rats may be in part due to the increased hepatic glucose production and prevailing peripheral insulin resistance. HFD-STZ induced type diabetic rats showed a significant development of insulin resistance compared to normal control rats. Carvacrol treatment decreases the insulin resistance and improves impaired glucose tolerance which is evident from the results of OGTT and HOMA-IR. Carvacrol administration augments insulin stimulated glucose uptake into peripheral tissues.

OGTT is the most common and more sensitive test measure for early abnormalities in glucose regulation than fasting plasma glucose or HbA1C. Intriguingly, glucose tolerance of HFD-STZ induced rats was significantly reduced and remained high even after 120 min of glucose loading. In contrast, in carvacrol treated diabetic rats, the blood glucose levels reached a peak and declined to fasting levels after 120 min. Impaired glucose tolerance reflects the hepatic gluconeogenesis and reduced uptake of glucose from blood by insulin dependent tissues.

The levels of plasma insulin were moderately decreased in HFD-STZ induced diabetic rats. Though the level was not decreased more, the insulin level in HFD-STZ diabetic rats could not facilitate glucose uptake due to insulin resistance. There was a improved insulin sensitivity and a rise in insulin level in carvacrol treated diabetic rats compared to the diabetic control rats suggesting that carvacrol exhibit significant insulin sensitization activity as well as improvement in the glucose homeostasis probably due to improved pancreatic β-cell function which is evident from improved plasma insulin levels.

Hyperglycemia is the clinical hallmark of poorly controlled diabetes which is known to cause glycation and also known as nonenzymatic glycosylation. In diabetes mellitus there is an increased level of glycosylated hemoglobin which is considered as a reliable index of glycemic control in diabetes. The glycation and subsequent browning (glycoxidation) reactions are enhanced by elevated glucose lev-}

els and there is some evidence that glycation itself may induce the formation of oxygen-derived free radicals. In the present study, administration of carvacrol for 30 days resulted in significant reduction of HbA1c thereby increasing the level of total hemoglobin in diabetic rats. This could be due to the result of improved glycemic control established by carvacrol.

Glycogen is a branched polymer of glucose residues which is synthesized by the enzyme glycogen synthase. The glycogen content, glycogen synthase activity and response to insulin signaling pathways are diminished and glycogen phosphorylase activity is significantly increased in diabetes mellitus. However, Carvacrol supplementation improved the levels of glycogen content in liver tissues of diabetic rats. This could be due to the insulin-sensitizing potential of carvacrol in the hepatic tissue.

Liver, the key organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants, and chemotherapeutic agents. AST, ALT, ALP, and GGT are the major hepatic marker enzymes. The elevation of hepatic markers in the serum is the result of leakage from damaged cells and, therefore, reflects the hepatocyte damage. Enzymes directly associated with the conversion of amino acids to keto acids are ALT and AST. ALT and AST activities are used as the indicators of hepatocyte damage. ALP, a membrane-bound glycoprotein enzyme, has been shown to be present in high concentration in the sinusoids and the endothelium of the central and periportal veins. Oral administration of carvacrol to diabetic group of rats showed a notable decline in the activity of these enzymes to their basal levels, indicating its non toxic as well as tissue protective nature.

Hyperlipidemia associated with insulin resistance is the clinical hallmark in the rats fed with high fat diet. Excessive intake of fatty acids results in accumulation of tissue triglycerides, particularly in fat tissues. Subsequently increased lipolysis and increases in circulating fatty acids are associated with rising adipocytes and lipolysis and insulin resistance. Fat distribution is a critical factor because insulin resistance known to be associated with visceral fat depots.

HFD-STZ induced rats showed abnormalities in lipid metabolism as evidenced from increased TG, TC, VLDL-c and LDL-c levels. Hypertriglyceridaemia observed in these HFD + STZ rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of TG-enriched hepatic VLDL and decreased TG uptake in peripheral tissues. Hypercholesterolaemia may be attributed to increased dietary cho-
lesterol absorption from the small intestine following the intake of HFD in a diabetic condition [33]. However, Oral carvacrol normalized the status of lipid profile indicating its antihyperlipidemic nature.

CONCLUSION
The results of the present study indicate that carvacrol exerted antihyperglycemic action which is evident from the results of OGTT and liver glycogen content. Carvacrol exhibits insulin sensitizing effect in HFD fed-STZ-induced diabetic rats which is evident from the results of HOMA-IR and plasma insulin level. The status of lipid profile was normalized upon carvacrol treatment indicating its antidyslipidemic nature. Hence, carvacrol can be considered for use in the treatment and management of T2DM. However, further studies are in progress to understand the exact mechanism of carvacrol in HFD-STZ induced T2D in experimental rats.

Conflict of Interest
The authors declare that there is no conflict of Interest.

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