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# Effect Of Pterostilbene On The Kidney Of C57BL/6J Mice With High Fat-Diet Induced Oxidative Stress

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**Keywords:**

Pterostilbene, etrahydrocurcumin, antioxidants, kidney markers, C57BL/6J mice.

**Abstract**

*Pterocarpus marsupium* has been used for many years in the treatment of diabetes mellitus. Pterostilbene (PTS) was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium*. Pterostilbene is a useful bioactive compound in preventing type 1 diabetes, insulin resistance and type 2 diabetes in animal models. Tetrahydrocurcumin (THC), one of the major metabolites of curcumin, exhibits many of the same physiologic and pharmacological activities as curcumin and in some systems may exert greater antioxidant activity than curcumin. The present study evaluated the protective effect of THC and PTS on high fat diet (HFD) induced diabetic mice. C57BL/6J mice were segregated in two groups, one fed standard diet (NC) and the other fed HFD for 16 weeks. Mice were fed continuously with high fat diet for 16 weeks and subjected to intragastric administration of THC (80 mg/kg body weight), PTS (40 mg/kg body weight) 9 to 16 weeks. At the end of the treatment nephritic markers, lipid peroxidation product, antioxidant examination was carried out to assess the efficacy of the treatment. HFD fed mice showed increased plasma glucose, insulin, altered nephritic markers, antioxidant. Oral Treatment with THC and PTS showed near normalized levels of plasma glucose, lipid peroxidation product, antioxidants, improved insulin and reduced kidney damage. THC administration showed more effective than PTS.

**Running title:** Effect of pterostilbene on the diabetes.

## Introduction

Diabetes mellitus is a complex metabolic disease characterized by high blood glucose levels and a disorder of carbohydrate, fat and protein metabolism. The abnormal increase of blood glucose in diabetes will result in long-term damage and dysfunction of various organs including the eyes, kidneys, nerves and blood vessels (American Diabetes Association, 2010; Murugan et al., 2023a). Diabetic nephropathy is a serious complication of type 1 diabetes and type 2 diabetes. It's also called diabetic kidney disease. In the United States, about 1 in 3 people living with diabetes have diabetic nephropathy. Diabetic nephropathy affects the kidneys' usual work of removing waste products and extra fluid from the body. The best way to prevent or delay diabetic nephropathy is by living a healthy lifestyle and keeping diabetes and high blood pressure managed. Over years, diabetic nephropathy slowly damages the kidneys' filtering system. Early treatment may prevent this condition or slow it and lower the chance of complications (Koya, et al., 2009; Murugan, 2021a).

Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances (Murugan, 2015a). *Pterocarpus marsupium* has been used for many years in the treatment of diabetes mellitus (Warrier et al., 1995). PTS was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium* (Murugan and Sakthivel, 2021) it is suggested that PTS might be one of the principal anti-diabetic constituents of Pterocarpus

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marsupium (Manickam et al., 1997). An aqueous extract of heartwood of P.marsupium has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients (ICMR, 1998).

THC was one of the major colourless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin (Murugan, 2015b). Curcumin was rapidly metabolized during absorption from the intestine, yielding THC (Murugan, 2023b), which had shown the strongest antioxidant activity among all curcuminoids (Murugan and Pari. 2007a). THC thought to play a pivotal role in protecting the cell membrane against lipid peroxidation, which exhibits its protective effect by means of □-diketone moieties and phenolic hydroxyl groups (Murugan, 2021b). Several studies in experimental animals indicated that THC also preventes cancer, protect the inflammation, atherosclerotic lesions and hepatotoxicity (Murugan et al., 2008).

**Materials and methods**

*Drugs and Chemicals*

THC and PTS was a gift provided by Sabinsa Corporation, USA. All other chemicals and biochemical were of analytical grade.

*Experimental animals*

Male C57BL/6J mice 3 weeks of age were obtained from NIN Hyderabad and housed in polypropylene cages. Animals were maintained under standard conditions with a 12h light/dark cycle. The animals received a standard pellet diet (Karnataka State Agro Corporation Ltd., Agro feeds division, Bangalore, India) and water *ad libitum*. After acclimatization for period of 1 week, mice were randomly divided into six groups.

*Experimental induction of diabetes*

The type 2 diabetes was induced through HFD. The standard diet which is commercially obtained from Sai Enterprises, Chennai, had a fat composition of 4.2%. The beef tallow based high fat diet was composed of protein - 17.7 g, fat - 35.2 g, carbohydrate - 34.5 g, fiber - 3.4 g, minerals - 6.8 g and vitamins -1.8g. Mice (6 nos.) from normal control group (group I) were fed standard diet for a period of 16 weeks. Mice from rest of the groups (group II-IV) were fed high fat diet for a period of 16 weeks. At the end of 8th week, the mice from all the groups were tested for blood glucose levels. Mice with blood glucose level of 220 mg/dl and above were considered to have developed insulin resistance and were subjected to intragastric administration of various doses of THC and PTS (as mentioned in the experimental design) during 8 to 16 weeks.

*Experimental design*

In this experiment, mice were divided into 4 groups of 6 mice each.

Group 1: Normal mice fed with a standard diet for 16 weeks

Group 2: HFD diabetic mice fed with high fat diet for a period of 16 weeks.

Group 3: HFD diabetic mice administered with THC (80 mg/kg body weight) by gavage for the last 8 weeks.

Group 4: HFD diabetic mice administered with PTS (40 mg/kg body weight) by gavage for the last 8 weeks.

At the end of experimental period mice were fasted overnight. The mice were sacrificed by cervical dislocation. Blood was collected by cutting the jugular vein into heparinized glass tubes. Plasma was obtained from blood samples after centrifugation (1500g for 10 min) and stored at 4°C. Kidney tissue was excised immediately from the mice and washed in ice-cold isotonic saline and blotted with a filter paper.

*Biochemical analysis*

Plasma glucose was estimated by the method of Trinder (1969). Plasma insulin was measured by the method of Burgi et al. (1988). Serum urea was estimated by the method of Fawcett and Scott (1960), uric acid by the enzymatic method described by Caraway (1955) and creatinine was estimated using the method of Tietz, 1987. Superoxide dismutase (SOD) in the kidney tissues was assayed by the method of Kakkar et al. (1984). The activity of catalase (CAT) in the kidney tissues was determined by the method of Sinha (1972). The activity of glutathione peroxidase (GPx) in the kidney tissues was measured by the method of Rotruck et al., (1973). Reduced glutathione (GSH) in the kidney tissues was estimated by the method of Ellman (1959). TBARS and Lipid hydroperoxide in kidney tissues were estimated by the methods of Niehaus and Samuelsson (1968) and Jiang et al., (1992), respectively.

*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).

**RESULTS**

Table 1 shows the level of blood glucose and plasma insulin of different experimental groups. HFD induced diabetic mice showed a significant increase in the level of blood glucose with significant decrease in the level of plasma insulin. Oral administration of THC and PTS to HFD induced diabetic mice significantly reversed the above biochemical changes. In agreement with these results, the present study also showed that the administration of THC and PTS significantly improved

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the blood glucose and plasma insulin levels. The THC administration showed more effective than PTS.

The level of urea, uric acid and creatinine in the serum of control and HFD induced diabetic mice are represented in the table 2. In our study, the levels of urea, uric acid and creatinine are elevated remarkably in the serum of diabetic mice as compared with control mice. Treatment of HFD induced diabetic mice with THC and PTS showed the reversed of these parameters near normal levels. The effect of THC was more potent than PTS.

Table 3 shows the changes in the levels of lipid peroxidation and the activities of antioxidant enzymes in normal and HFD induced diabetic mice. TBARS and hydroperoxides (LOOH) from kidney homogenate were significantly decreased with THC treatment whereas; HFD induced diabetic mice PTS showed significantly increased levels of lipid peroxidation products. The effect of THC was better than PTS.

For studying the effect of THC and PTS on free radical production, the activities of SOD, CAT, GPx and GSH were measured (Table 4). They presented significant increases in THC and PTS treatment when compared with HFD induced diabetic mice. The effect of THC was more prominent compared with PTS.

**Discussion**

Oxidative stress in cells and tissues results from the increased generation of free radicals and/or from decreases in antioxidant potential (Murugan and Jawahar, 2020). Elevated generation of free radicals resulting in the consumption of antioxidant defence components may lead to disruption of cellular function and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation (Hunt et al. 1988). Oxidative stress has been suggested to play a key role in the pathogenesis of these complications (Ceriello, 2000; Murugan and Pari, 2006). Several mechanisms have been put forth to explain the genesis of the free radical in diabetes. These include autoxidation processes of glucose, the non-enzymatic glycation of proteins with the consequently increased formation of AGEs and enhanced glucose flux through the polyol pathway (Elgawish et al. 1996).

Under physiological conditions, a wide range of antioxidant defenses protects against the adverse effects of free radical production in vivo. Hyperglycemia causes release of tissue damaging reactive oxygen species (ROS) and alters the balance between radical production and protective antioxidant defense (Signorini et al. 2002). The level of lipid peroxidation in cell is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger systems, the levels of which are altered in diabetes (Wohaieb and Godin, 1987). Any compound natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this damage may have a significant role in treatment of diabetes mellitus. The present section provides the antiperoxidative and antioxidant effect of THC and PTS in HFD induced diabetic mice.

In general, creatinine level is considered to assess kidney function (Kern et al., 2010; Murugan et al., 2020; Murugan and Pari, 2007b). Elevated levels of serum creatinine, urea and uric acid were observed in diabetic kidney. Blood urea is produced as a result of protein breakdown and formed in the liver which carried via the bloodstream to the kidneys to be eliminated. Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. Uric acid inside the sample is oxidized by using uricase to allantoin. The assay of creatinine turned into based totally on the response of creatinine with alkaline picrate. Creatinine is a breakdown product formed in certain muscular tissues and carried thru the bloodstream and eliminated by way of the kidneys. If the kidneys dysfunction, they are unable to dispose of the usual amount of these materials and as an end result, the blood urea and creatinine stages will enhance (Myers et al., 2010). Moreover, these elevations were found to be associated with interstitial atrophy, epithelial necrosis as well as atrophic changes in glomeruli, and thus DN (Yokozawa et al., 2005). Diabetic mice showed significant increase in serum creatinine, urea and uric acid levels which were significantly reduced after treatment with THC and PTS indicating recovery toward normal level.

TBARS and LOOH significantly increased in kidney of diabetic control mice. Previous studies have also reported that there was an increased lipid peroxidation in plasma of diabetic rats (Murugan and Pari, 2006b; Pari and Murugan, 2007). The increase in oxygen free radicals in diabetes could be due to rise in blood glucose levels, which upon autoxidation generate free radicals.

Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. Aim our study, the diabetic tissues showed a decrease in the activities of key antioxidants like SOD, CAT, GSH, GPx, GSH which play an important role in scavenging the toxic intermediate of incomplete oxidation. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals *in vivo*. Previous studies have reported that the activity of SOD is low in diabetes mellitus (Feillet-Coudray et al. 1999). A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion $O_2^{\bullet-}$ and hydrogen peroxide in biological systems, which inturn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation (Kumuhekar and Katyane, 1992).

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The depletion of GSH, GPx promotes generation of ROS and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes (Raza et al. 2000). As the alteration produced in the antioxidant activities indicate the involvement of deleterious oxidative changes, increased activities of the components of this defence system would therefore be important in protection against radical damage.

It may be concluded that in diabetes, kidney tissue was more vulnerable to oxidative stress and showed increased lipid peroxidation. The above observations showed that THC and PTS possesses antioxidant effect that may contribute to its protective action against kidney markers, lipid peroxidation and enhancement of cellular antioxidant defense. This activity contributes to the protection against oxidative damage in HFD induced diabetic mice. THC administration showed more effective than PTS.

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**Table 1. Effect of THC on the levels of blood glucose, plasma insulin in normal and experimental mice**

| Groups | Fasting blood glucose (mg/dl) | Plasma insulin (μU/ml) |
|---|---|---|
| NC | $98.52 \pm 4.28^{a}$ | $12.31 \pm 0.72^{a}$ |
| Diabetic control | $280.57 \pm 7.47^{b}$ | $3.90 \pm 0.21^{b}$ |
| Diabetic + THC (80 mg/kg) | $115.45 \pm 6.52^{c}$ | $10.12 \pm 0.50^{c}$ |
| Diabetic + Pterostilbene (40mg/kg) | $129.15 \pm 8.51^{d}$ | $8.75 \pm 0.40^{d}$ |

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).

**Table 2. Effect of THC and PTS on urea, uricacid, creatinine of serum in experimental mice**

| Group | Urea (mg/dL) | Uricacid(mg/dL) | Creatinine(mg/dL) |
|---|---|---|---|
| **NC** | $22.15 \pm 1.30^{a}$ | $1.40 \pm 0.07^{a}$ | $0.92 \pm 0.04^{a}$ |
| **HFD** | $51.40 \pm 2.77^{b}$ | $3.99 \pm 0.17^{b}$ | $3.25 \pm 0.20^{b}$ |
| **HFD + THC(80 mg/kg body weight)** | $27.25 \pm 2.21^{c}$ | $1.70 \pm 0.16^{c}$ | $1.12 \pm 0.07^{c}$ |
| **HFD + PTS (40 mg/kg body weight)** | $32.28 \pm 1.56^{d}$ | $2.35 \pm 0.10^{d}$ | $1.55 \pm 0.06^{d}$ |

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Values that have a different superscript letter (a,b,c,d) differ significantly with each other ($p < 0.05$, DMRT).

**Table 3. Effect of THC and PTS on TBARS and LOOH in the kidney of HFD-fed C57BL/6Jmice**

| Group | TBARS | LOOH |
|---|---|---|
| **Normal** | $1.47 \pm 0.07^{a}$ | $67.70 \pm 3.75^{a}$ |
| **HFD** | $3.92 \pm 0.15^{b}$ | $150.21 \pm 9.30^{b}$ |
| **HFD+ THC (80 mg/kg body weight)** | $1.51 \pm 0.08^{c}$ | $72.54 \pm 5.67^{c}$ |
| **HFD+PTS (40 mg/kg body weight)** | $1.98 \pm 0.07^{d}$ | $85.09 \pm 4.60^{d}$ |

Values that have a different superscript letter (a,b,c,d) differ significantly with each other ($p < 0.05$, DMRT).

**Table 3. Effect of THC and PTS on the activities of antioxidant in the kindey of diabetic and normal mice**

| Group | NC | HFD | HFD+THC (80 mg/kg body weight) | HFD+PTS (40 mg/kg body weight) |
|---|---|---|---|---|
| SOD (U*/mgprotein) | $12.52 \pm 1.01^{a}$ | $7.78 \pm 0.56^{b}$ | $11.45 \pm 0.77^{c}$ | $9.32 \pm 0.70^{d}$ |
| CAT ($U^{\#}$/mgprotein) | $36.54 \pm 2.51^{a}$ | $19.55 \pm 1.20^{b}$ | $30.74 \pm 2.11^{c}$ | $26.25 \pm 1.41^{d}$ |
| GPX ($U^{\square}$/mgprotein) | $10.21 \pm 0.69^{c}$ | $4.97 \pm 0.31^{c}$ | $8.78 \pm 0.45^{c}$ | $7.05 \pm 0.63^{c}$ |
| GSH (µg/mgprotein) | $12.09 \pm 0.78^{d}$ | $6.34 \pm 0.38^{d}$ | $11.89 \pm 0.89^{c}$ | $9.74 \pm 0.62^{d}$ |

U*=enzyme concentration required to inhibit the NBT to 50% reduction in one minute.
$U^{\#}$ = □mole of $H_2O_2$ consumed/minute. $U^{\square}$ = □g of GSH utilized/minute.
Values that have a different superscript letter (a, b, c, d) differ significantly with each other ($p < 0.05$, DMRT).