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Original Article

# Effects of pterostilbene on brain lipid peroxidation in diabetic rats

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## ABSTRACT

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. Oxidative stress has been suggested to be a contributory factor in the development and complication of diabetes. In the present study, the effect of tetrahydrocurcumin (THC), one of the active metabolites of curcumin, on the antioxidant status of streptozotocin-nicotinamide-induced diabetic rats was investigated. The effect of THC compared to pterostilbene on the occurrence of oxidative stress in the brain of rats during diabetes was also investigated by measuring the extent of oxidative damage as well as the status of the antioxidant defense system. Oral administration of THC at 80 mg/kg and pterostilbene at 40 mg/kg body weight of diabetic rats for 45 days resulted in significant reduction in blood glucose and significant increase in plasma insulin levels. Oxidative stress results from an imbalance between the generation of oxygen-derived radicals and the organism's antioxidant potential. Thiobarbituric acid reactive substances (TBARS) and hydroperoxides from brain of diabetic rats were found to significantly increase and enzymatic antioxidants, namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) and non-enzymatic antioxidants, namely vitamin C, vitamin E and reduced glutathione (GSH) were found to significantly decrease in diabetic control rats. Administration of THC and pterostilbene significantly decreased the levels of TBARS and hydroperoxides accompanied with a significant increase in the activities of enzymatic antioxidants as well as non-enzymatic antioxidants. The anti-diabetic and antioxidant effects of THC are more potent than those of pterostilbene.

**KEYWORDS:** tetrahydrocurcumin, pterostilbene, lipid peroxidation, antioxidants, streptozotocin, diabetes.

## INTRODUCTION

Diabetes mellitus is a common metabolic disorder which is associated with chronic complications such as nephropathy, angiopathy, retinopathy, and peripheral neuropathy. However, as early as 1922 it was recognized that diabetes also can lead to cognitive dysfunction [1]. Since then, studies in experimental models and in patients recorded alterations in neurotransmission, electrophysiological and structural abnormalities, and neurobehavioral alterations, in particular cognitive dysfunction and increased risk of depression [2]. The major complications of hyperglycemia also include the potentiation of neuronal damage observed following hypoxic/ischemic events, as well as stroke. Glucose utilization has been shown to decrease in the brain during diabetes [3], confirming a potential mechanism for increased vulnerability to acute pathological events.

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Diabetes mellitus, a state of chronic hyperglycemia, is a common disease affecting over 124 million individuals worldwide [4]. Type 2 diabetes has been associated with increased oxidative stress [5]. Free radicals are continually produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Under physiological conditions, a wide range of antioxidant defenses protects against adverse effects of free radical production *in vivo* [6]. Oxidative stress results from an imbalance between radical production and reduced activity of antioxidant defenses or both these phenomena. Similarly, oxidative damage in rat brain has been shown to increase by experimentally induced hyperglycemia [7]. Under experimental conditions, hyperglycemia dramatically increases neuronal alterations and glial cell damage caused by temporary ischemia [8].

Hyperglycemia causes an imbalance in the oxidative status of the nervous tissue and the resulting free radicals damage the brain through a peroxidative mechanism [8]. It has been proposed that streptozotocin acts as a diabetogenic agent owing to its ability to destroy pancreatic β-cells, possibly by a free radical mechanism [9]. The level of lipid peroxidation in cell is controlled by various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavenger systems, the levels of which are altered in diabetes [10].

Diabetes mellitus is becoming a pandemic and despite the recent upsurge in new drugs to treat and prevent the condition, its prevalence continues to soar. Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease-related complications are increasing unabated. Parallel to this, recent developments in understanding the pathophysiology of the disease process have opened up several new avenues to identify and develop novel therapies to combat the diabetic plaque [11].

Therefore, as the disease is progressing unabated, there is a need for identifying indigenous natural resources in order to procure them and study in detail their potential on different newly identified targets so as to develop them as new therapeutics [12]. Currently available drug regimens for the management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective anti-diabetic drugs. The belief that natural medicines are much safer than synthetic drugs has gained popularity in recent years and has led to tremendous growth in phytopharmaceutical usage [13].

Several medicinal phytochemicals such as tetrahydrocurcumin [14], *Curuma longa* [15], *Cassia auriculata* [16], etc. have been reported to possess anti-hyperglycemic effects. The attributed anti-hyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to facilitate metabolites in insulin dependent processes. Hence treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels [17]. In general, there is very little biological knowledge on their specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently implicated as having anti-diabetic effects [18].

THC is one of the major colorless metabolites of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin [19]. Curcumin was rapidly metabolized during absorption from the intestine, yielding THC [20], which had shown the strongest antioxidant activity among all curcuminoids [21]. THC is thought to play a pivotal role in protecting the cell membrane against lipid peroxidation, which exhibits its protective effect by means of β-diketone moieties and phenolic hydroxyl groups [22]. Several studies in experimental animals indicated that THC also prevents cancer, and protect from inflammation, atherosclerotic lesions and hepatotoxicity [23].

Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of *P. marsupium*. The water stored in tumblers made out of the heartwood of *P. marsupium* is used as a traditional therapy for patients with diabetes mellitus [24]. An aqueous extract of heartwood of *P. marsupium* has been tested clinically and found to be effective in NIDDM patients (ICMR, 1998). When administered to STZ-induced hyperglycemic rats, pterostilbene and marsupin two of the major phenolic constituents in aqueous decoction of the heartwood of *P. marsupium*, significantly decreased plasma glucose [25].

To the author's knowledge, so far no other biochemical investigation has compared the effect of THC and pterostilbene on the antioxidant status of brain in experimental diabetic rats. The present investigation was carried out to study the effect of THC and pterostilbene on brain lipid peroxides and antioxidants in rats with STZ and nicotinamide-induced diabetes.

## MATERIALS AND METHODS

### Drugs and chemicals

THC and pterostilbene was gifted by Sabinsa Corporation, USA. All other chemicals and biochemicals were of analytical grade.

### Induction of diabetes

Non-insulin dependent diabetes mellitus was induced [26] in overnight-fasted rats by a single intraperitonial injection (i.p) of STZ (65 mg/kg body weight), 15 min after the i.p administration of nicotinamide (110 mg/kg body weight). 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 were used for the study.

### Experimental design

In the experiment, a total of 24 rats (18 diabetic surviving rats, 6 normal rats) were used. The rats were divided into four groups of six each after the induction of STZ diabetes: group I: normal untreated rats, group II: diabetic control rats, group III: diabetic rats given THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days [20] and group IV: diabetic rats given pterostilbene (40 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days [23]. The experimental period was 45 days.

Animals were sacrificed at the end of 45 days by cervical dislocation. Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose, and plasma was separated for assay of insulin. The entire brain was perfused immediately with ice-cold 0.9% sodium chloride. Thiobarbituric acid reactive substances (TBARS), hydroperoxides, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione were estimated in brain [24].

### Analytical procedures

#### Measurement of blood glucose and plasma insulin

Blood glucose was determined by the O-toluidine method [25]. Plasma insulin was assayed by the enzyme- linked immunosorbent assay method using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany).

### Estimation of lipid peroxidation

Lipid peroxidation in liver and kidney was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides using the methods of Fraga *et al*. [26] and Jiang *et al*. [27], respectively. In brief, 0.1 ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid-HCl reagent (0.37% TBA, 0.25 M HCl and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at 3500 X g for 10 min at room temperature and the absorbance of clear supernatant was measured at 535 nm against a reference blank. Values were expressed as mM/100g tissue.

Hydroperoxides were expressed as mM/100 g tissue. Tissue homogenate (0.1 ml) was treated with 0.9 ml of Fox reagent (88 mg of butylated hydroxy toluene (BHT), 7.6 mg of xylenol orange and 0.8 mg of ammonium iron sulphate were added to 90 ml of methanol and 10 ml of 250 mM sulphuric acid) and incubated at 37 °C for 30 min. Then the absorbance was read at 560 nm.

### Estimation of catalase activity

Catalase (CAT*)* was estimated by the method of Sinha [28]. The reaction mixture (1.5 ml, vol) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2M $H_2O_2$. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then the absorbance was read at 620 nm; CAT activity was expressed as μM of $H_2O_2$ consumed/min/mg protein.

**Estimation of superoxide dismutase (SOD) activity**

The activity of SOD was assayed by the method of Kakkar *et al*. [29]. 0.5 ml of tissue homogenate was diluted with 1 ml of water. In this mixture, 2.5 ml of ethanol and 1.5 ml of chloroform (all reagents chilled) were added and shaken for 1 min at 4 °C and then centrifuged. The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 ml of 186 μM PMS, 0.3 ml of 30 μM NBT, 0.2 ml of 780 μM NADH, appropriately diluted enzyme preparation and water in a total volume of 3 ml. Reaction was started by the addition of NADH. After incubation at 30 °C for 90 sec the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560 nm against butanol blank. A system devoid of enzyme served as the control. One unit of the enzyme activity is defined as the enzyme reaction which caused 50% inhibition of NBT reduction in one minute under the assay conditions.

**Estimation of glutathione peroxides (GPx) activity**

GPX activity was measured by the method described by Rotruck *et al*. [30]. Briefly, the reaction mixture contained 0.2 ml 0.4M phosphate buffer (pH 7.0), 0.1 ml 10 mM sodium azide, 0.2 ml tissue homogenized in 0.4M phosphate buffer (pH 7.0), 0.2 ml glutathione, and 0.1 ml 0.2 mM hydrogen peroxide. The contents were incubated for 10 min at 37 °C. 0.4 ml 10% TCA was added to stop the reaction and centrifuged at 3200 X g for 20 min. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid (DTNB) in 100 ml 0.1% sodium nitrate). The activities were expressed as μg of GSH consumed/min/mg protein.

**Estimation of glutathione-S-transferase (GST) activity**

GST activity was determined spectrophotometrically by the method of Habig *et al*. [31]. The reaction mixture contained 1.0 ml 100 mM phosphate buffer (pH 6.5), 0.1 ml 30 mM 1-chloro-2, 4-dinitrobenzene (CDNB), and 0.7 ml double distilled water. After pre-incubating the reaction mixture for 5 min at 37 °C, the reaction was started by the addition of 0.1 ml tissue homogenate and 0.1 ml of glutathione as substrate. After 5 min the absorbance was read at 340 nm. Reaction mixture without the enzyme was used as a blank. The activity of GST was expressed as mM of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6/ mM/cm.

**Estimation of reduced glutathione (GSH)**

GSH was determined by the method of Ellman [32]. A known weight of tissue was homogenized in phosphate buffer. From this 0.5 ml was pipetted out and precipitated with 2 ml of 5% TCA. 1 ml of the supernatant was taken after centrifugation at 3200 X g for 20 min and 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer were added (pH 8.0). Then the absorbance was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer. The values were expressed as mg/100 g tissue.

**Histopathological study**

The liver, kidney and pancreas samples fixed for 48 h 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 μm thick) were prepared and then stained with hematoxylin and eosin dye, and 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 $p < 0.05$ [33].

## RESULTS

**Plasma glucose**

Table 1 shows the effect of pterostilbene and metformin on glucose levels at 0, 2, 4 and 6 weeks. In the pterostilbene-treated groups (all doses), although a significant anti-hyperglycemic effect was evident from the second week onwards, the decrease in glucose was maximum (56.54%) by

the end of sixth week in the group receiving 40 mg/kg body weight of pterostilbene. Based on these data, the higher dose, 40 mg/kg body weight of pterostilbene, was selected for further evaluation.

### Plasma insulin, hemoglobin, glycated hemoglobin and urine sugar

Table 2 presents the levels of plasma insulin, total hemoglobin, glycated hemoglobin and urine sugar of normal control and experimental rats. There was a significant elevation in glycated Hb levels, whereas plasma insulin and total hemoglobin levels decreased significantly in diabetic rats when compared with normal rats. In diabetic control rats, urine sugar was more than 2%, but in the case of rats treated with pterostilbene and THC, there was no urine sugar. Oral administration of THC and pterostilbene to diabetic rats significantly restored total hemoglobin and glycosylated hemoglobin levels.

### Effect on brain lipid peroxidation and antioxidants

Table 3 shows the changes in the levels of lipid peroxidation and the activities of antioxidant enzymes in normal and experimental rats. TBARS and hydroperoxides from brain homogenate significantly decreased with THC treatment whereas diabetic control rats showed significantly increased

**Table 1.** Changes in the levels of glucose in control and experimental rats at 2, 4 and 6 weeks.

| Groups | Glucose (mg/dl) | | | |
|---|---|---|---|---|
| | 0 day | 2nd week | 4th week | 6th week |
| Normal control | 73.31 ± 5.48 | 80.46 ± 5.38$^{a}$ | 77.6 ± 7.58$^{a}$ | 74.61 ± 5.41$^{a}$ |
| Normal + Pterostilbene (40 mg/kg) | 81.61 ± 6.56 | 77.41 ± 5.61$^{a}$ | 72.65 ± 6.58$^{a}$ | 69.51 ± 4.57$^{a}$ |
| Diabetic control | 315.35 ± 24.21 | 325.45 ± 27.31$^{b}$ | 362.75 ± 29.55$^{b}$ | 397.31 ± 33.56$^{b}$ |
| Diabetic + Pterostilbene (10 mg/kg) | 291.54 ± 25.85 | 265.35 ± 24.58$^{c}$ | 234.20 ± 20.55$^{c}$ | 185.75 ± 14.58$^{c}$ |
| Diabetic + Pterostilbene (20 mg/kg) | 285.58 ± 22.32 | 245.58 ± 22.31$^{cd}$ | 206.31 ± 15.62$^{c}$ | 167.21 ± 13.41$^{d}$ |
| Diabetic + Pterostilbene (40 mg/kg) | 278.47 ± 24.55 | 225.25 ± 18.33$^{d}$ | 177.41 ± 13.77$^{d}$ | 124.41 ± 11.02$^{e}$ |
| Diabetic + THC (80 mg/kg) | 284.41 ± 23.41 | 251.21 ± 21.01$^{c}$ | 196.41 ± 17.35$^{c}$ | 141.75 ± 11.21$^{f}$ |

Values are mean ± SD for 6 rats in each group. $^{a\text{-}f}$: In each column, means with different superscript letters differ significantly at $p<0.05$ (DMRT).

**Table 2.** Changes in the levels of insulin, hemoglobin, glycosylated hemoglobin and urine sugar in control and experimental rats.

| Groups | Insulin (μU/ml) | Haemoglobin (g/dl) | Glycosylated Hb (mg/gHb) | Urine sugar |
|---|---|---|---|---|
| Normal control | 16.41 ± 1.32$^{a}$ | 11.55 ± 0.92$^{a}$ | 0.25 ± 0.02$^{a}$ | Nil |
| Normal + Pterostilbene (40 mg/kg) | 17.31 ± 1.41$^{a}$ | 12.54 ± 0.71$^{a}$ | 0.24 ± 0.02$^{a}$ | Nil |
| Diabetic control | 6.35 ± 0.48$^{b}$ | 9.05 ± 0.60$^{b}$ | 0.50 ± 0.04$^{b}$ | +++ |
| Diabetic + Pterostilbene (40 mg/kg) | 14.31 ± 1.49$^{c}$ | 10.58 ± 0.68$^{c}$ | 0.34 ± 0.02$^{c}$ | Nil |
| Diabetic + THC (80 mg/kg) | 11.39 ± 0.48$^{c}$ | 10.54 ± 0.78$^{c}$ | 0.37 ± 0.03$^{c}$ | Trace |

Values are mean ± SD for 6 rats in each group. $^{a\text{-}c}$: In each column, means with different superscript letters differ significantly at $p<0.05$ (DMRT). + + +: > 2% sugar

**Table 3.** Changes in the levels of TBARS, hydroperoxides, catalase, superoxide dismutase, glutathione peroxidase, glutathione-s-transferase and reduced glutathione in the brain of normal and experimental animals.

| Groups | Normal | Diabetic control | Diabetic + Pterostilbene (40 mg/kg) | Diabetic + THC (80 mg/kg) |
|---|---|---|---|---|
| **TBARS** | | | | |
| Brain (mM/100g tissue) | $1.16 \pm 0.05^{a}$ | $1.84 \pm 0.11^{b}$ | $1.39 \pm 0.07^{d}$ | $1.23 \pm 0.06^{c}$ |
| **Hydroperoxides** | | | | |
| Brain (mM/100g tissue) | $118.01 \pm 5.78^{a}$ | $137.78 \pm 7.56^{b}$ | $129.32 \pm 5.43^{d}$ | $123.67 \pm 5.29^{c}$ |
| **Catalase** | | | | |
| Brain (Units$^{A}$ / mg protein) | $3.28 \pm 0.15^{a}$ | $0.86 \pm 0.04^{b}$ | $1.96 \pm 0.11^{d}$ | $2.76 \pm 0.15^{c}$ |
| **Superoxide dismutase** | | | | |
| Brain (Units$^{B}$ / mg protein) | $7.63 \pm 0.43^{a}$ | $5.21 \pm 0.34^{b}$ | $6.28 \pm 0.33^{d}$ | $7.21 \pm 0.42^{c}$ |
| **Glutathione peroxidase** | | | | |
| Brain (Units$^{C}$ / mg protein) | $3.39 \pm 0.20^{a}$ | $1.05 \pm 0.07^{b}$ | $1.99 \pm 0.10^{d}$ | $2.89 \pm 0.18^{c}$ |
| **Glutathione-S-transferase** | | | | |
| Brain (Units$^{D}$ / mg protein) | $5.76 \pm 0.35^{a}$ | $0.83 \pm 0.04^{b}$ | $2.05 \pm 0.11^{d}$ | $2.82 \pm 0.16^{c}$ |
| Reduced Glutathione | | | | |
| **Brain (mg / 100g tissue)** | $34.42 \pm 2.22^{a}$ | $14.84 \pm 1.01^{b}$ | $23.34 \pm 1.31^{d}$ | $30.22 \pm 1.88^{c}$ |

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).
A - μ moles of $H_2O_2$ consumed/minute.
B – One unit of activity was taken as the enzyme reaction which caused 50% inhibition of NBT reduction in one minute.
C - μg of GSH consumed/min.
D - μ moles of CDNB-GSH conjugate formed/min.

levels of lipid peroxidation products. The effect of THC was better than pterostilbene.

For studying the effect of THC and pterostilbene on free radical production, the activities of SOD, CAT, GPx, GST and GSH were estimated. They presented significant increases as a result of THC treatment when compared with diabetic control rats. The effect of THC was more prominent compared with pterostilbene.

### Histopathological observations in liver, kidney and pancreas of normal and experimental rats

### Liver

Histopathological section of normal rat liver is shown in Fig. 1A. Histopathological section of diabetic control rat liver showed portal triad with mild inflammation and cell infiltration, sinusoidal congestion and fatty degeneration in the form of fat lake and fatty change predominantly of micro-vesicular type (Fig. 1B-D). Diabetic rats treated with 10 and 20 mg/kg body weight of pterostilbene revealed focal granuloma with macro-vesicular fatty generation and mild sinusoidal dilatation and congestion (Fig. 1E and F). Treatment

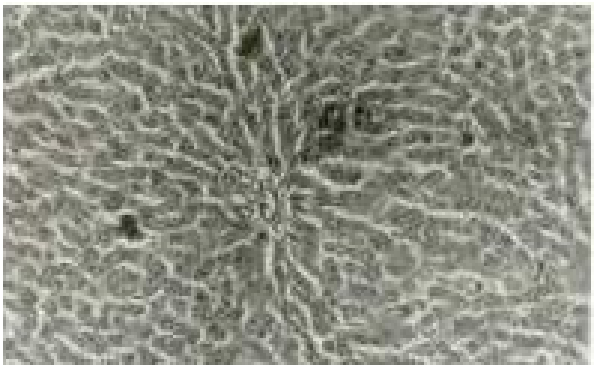

**Fig. 1A.** Normal rat liver (H&E x 20).

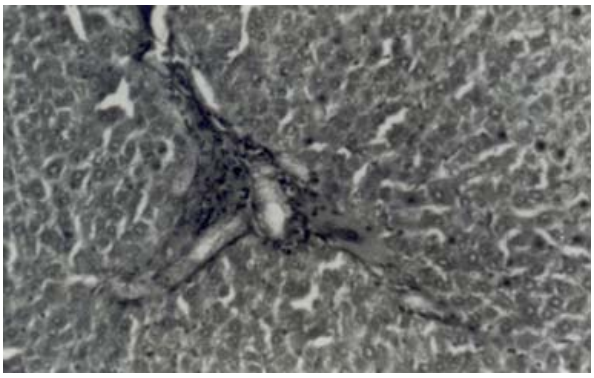

**Fig. 1B.** Diabetic control rat liver (H&E x 20). Portal triad with mild inflammation and cell infiltration.

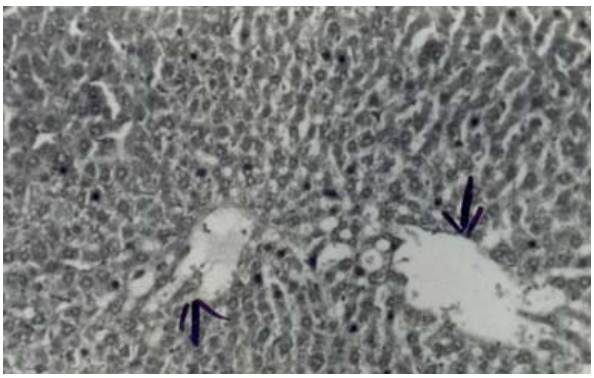

**Fig. 1C.** Diabetic control rat liver (H&E x 20). Sinusoidal congestion and fatty degeneration in the form of fat lake (→).

of diabetic rats with 40 mg/kg body weight of pterostilbene documented mild portal inflammation (Fig. 1G). Administration of 80 mg/kg body weight of THC to diabetic rats showed sinusoidal dilatation and focal Kupffer cell hyperplasia (Fig. 1H).

Doses of 10 and 20 mg/kg of pterostilbene caused a decrease in the glycaemia; however, they showed liver damage at lower doses, which may be due to decreased effect of pterostilbene in protecting the tissue against STZ-induced toxicity. 40 mg/kg of pterostilbene showed reduced liver damage.

**Kidney**

Normal rat kidney is shown in Fig. 2A. Diabetic control rat kidney showed fatty infiltration,

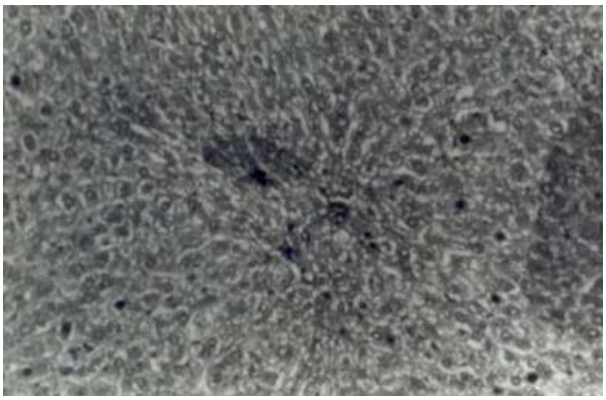

**Fig. 1D.** Diabetic control rat liver (H&E x 20). Another area of fatty change, predominantly microvesicular.

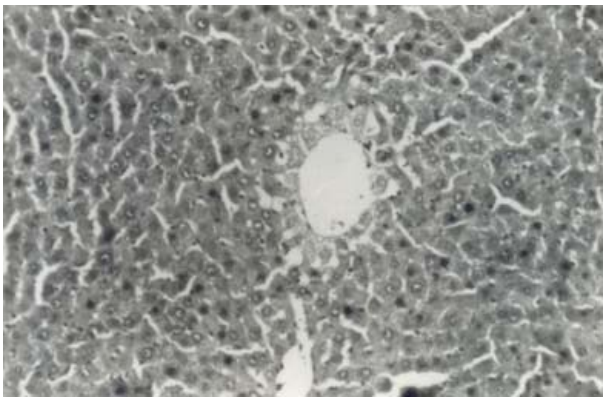

**Fig. 1E.** Liver of diabetic rats treated with pterostilbene (10 mg) (H&E x 20). Focal granuloma and macrovesicular fatty degeneration.

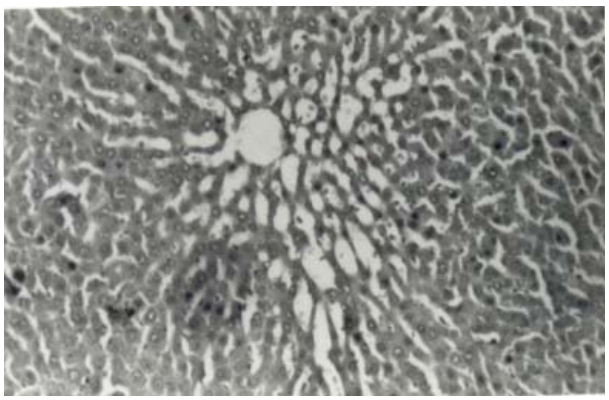

**Fig. 1F.** Liver of diabetic rats treated with pterostilbene (20 mg) (H&E x 20). Mild sinusoidal dilatation and congestion.

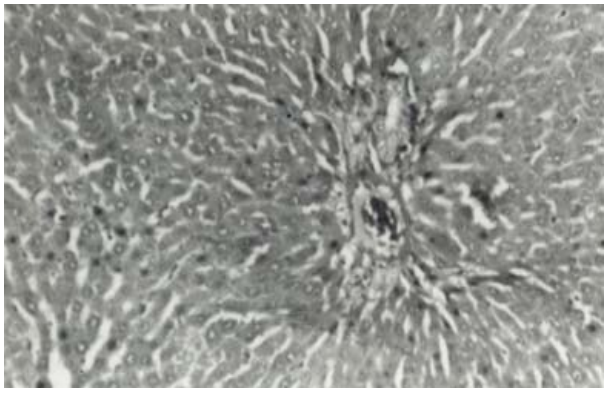

**Fig. 1G.** Liver of diabetic rats treated with pterostilbene (40 mg) (H&E x 20). Mild portal inflammation with near normal appearance.

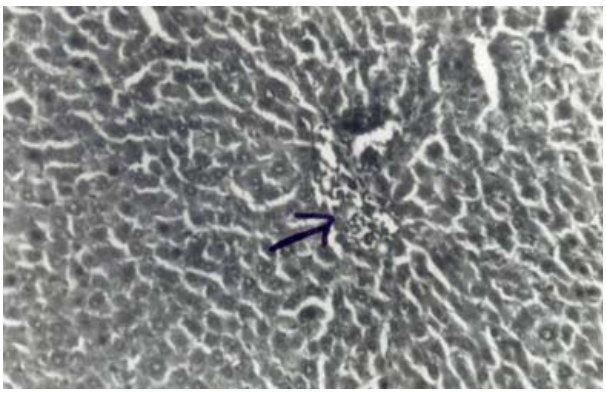

**Fig. 1H.** Liver of diabetic rats treated with THC (80 mg) (H&E x 20). Mild sinusoidal dilatation and focal Kupffer cell hyperplasia.

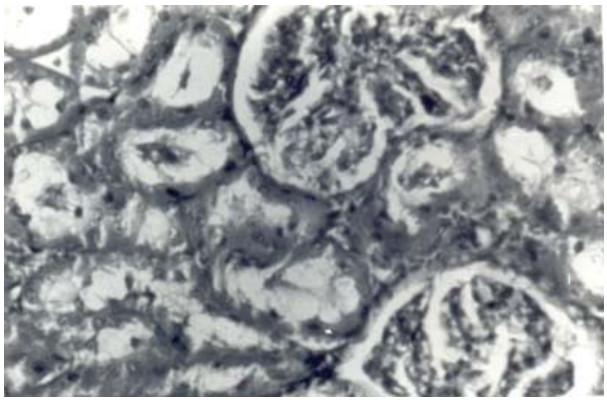

**Fig. 2A.** Normal rat kidney (H&E x 20).

parenchymal inflammation and hemorrhages (Fig. 2B-D). Diabetic rats treated with 10, 20 and 40 mg/kg of pterostilbene revealed parenchymal inflammation, fatty infiltration, mild parenchymal

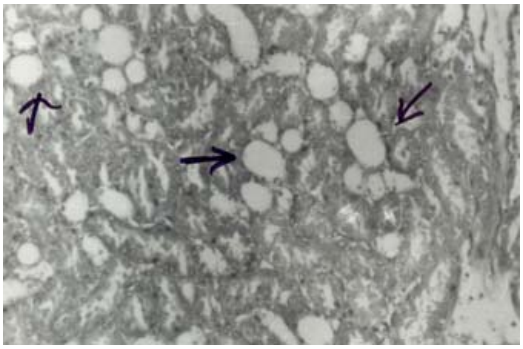

**Fig. 2B.** Diabetic control rat kidney (H&E x 20). Fatty infiltration (→).

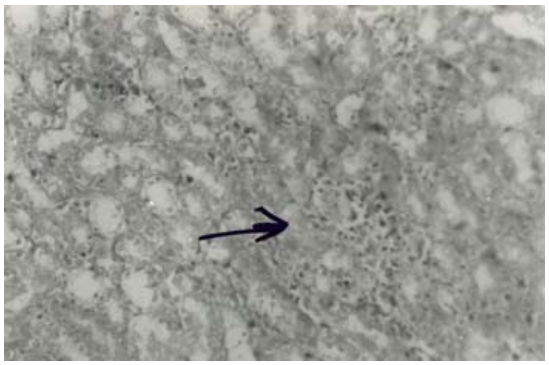

**Fig. 2C.** Diabetic control rat kidney (H&E x 20). Parenchymal inflammation (→).

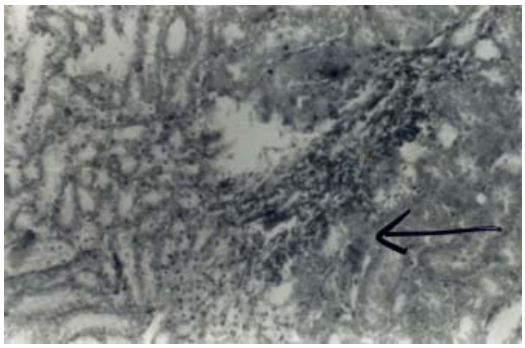

**Fig. 2D.** Diabetic control rat kidney (H&E x 20). Hemorrhages (→).

inflammation and necrotic areas (Fig. 2E, F, G and H), whereas treatment with 80 mg/kg THC showed cloudy swelling of tubules (Fig. 2I).

The histological evidence of diabetic control rats suggests that structural alterations at the end of 3 weeks are due to STZ-induced free radical generation quite early in diabetes. Damage to the kidney was significantly reduced in diabetic rats treated with 200 mg/kg of pterostilbene. Pterostilbene at 10, 20 and 40 mg/kg caused damage to kidney of diabetic rats whereas 40 mg/kg of pterostilbene reduced the toxic effects of STZ.

**Pancreas**

Normal rat pancreas with β-islets is shown in Fig. 3A. Diabetic control rat pancreas showed fatty

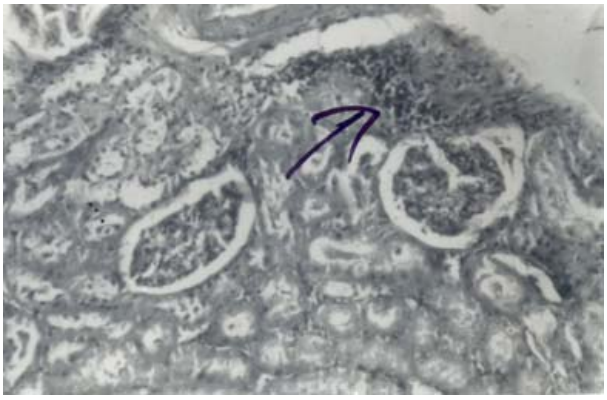

**Fig. 2G.** Kidney of diabetic rats treated with pterostilbene (40 mg) (H&E x 20). Mild parenchymal inflammation (→).

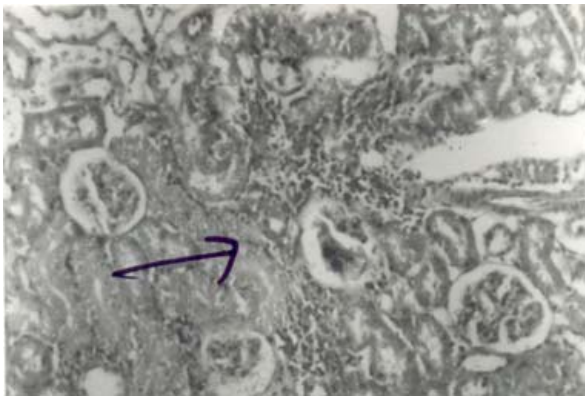

**Fig. 2E.** Kidney of diabetic rats treated with pterostilbene (10 mg) (H&E x 20). Parenchymal inflammation (→).

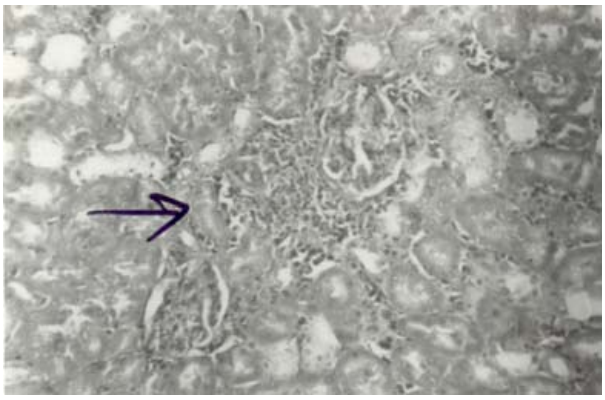

**Fig. 2H.** Kidney of diabetic rats treated with pterostilbene (40 mg) (H&E x 20). Parenchymal inflammation and necrotic areas (→).

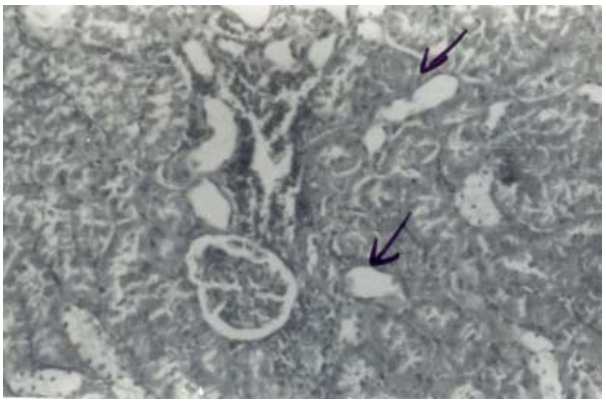

**Fig. 2F.** Kidney of diabetic rats treated with pterostilbene (20 mg) (H&E x 20). Fatty infiltration (→).

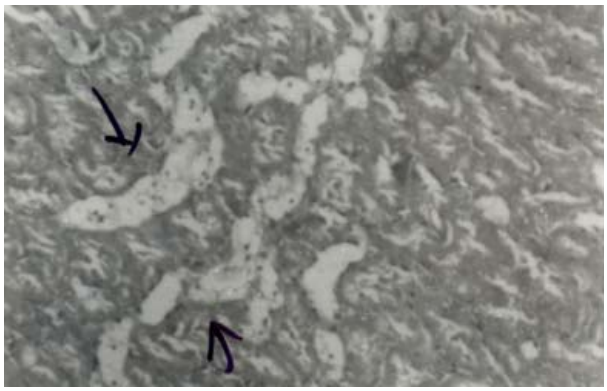

**Fig. 2I.** Kidney of diabetic rats treated with THC (80 mg) (H&E x 20). Cloudy swelling of tubules (→).

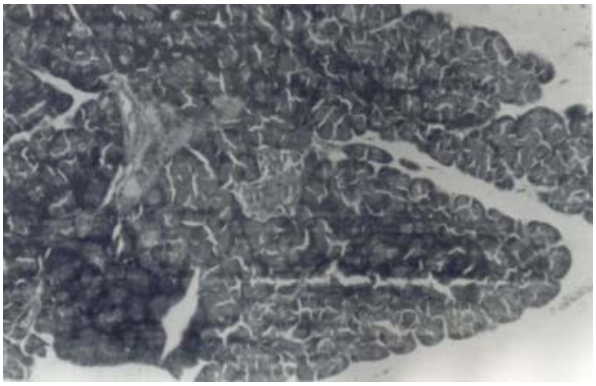

**Fig. 3A.** Normal rat pancreas (H&E x 20) showing β-islets.

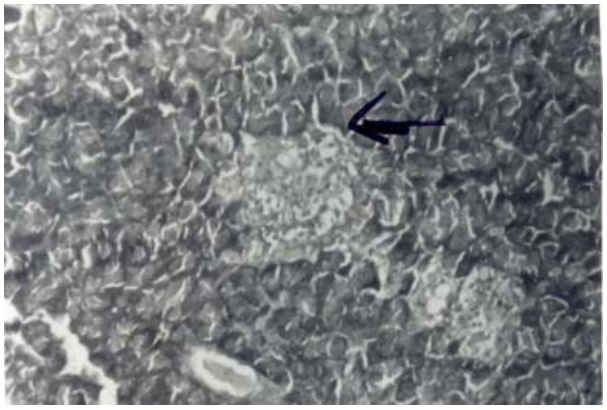

**Fig. 3B.** Diabetic control rat pancreas (H&E x 20). Fatty infiltration of islet cells and shrinkage (→).

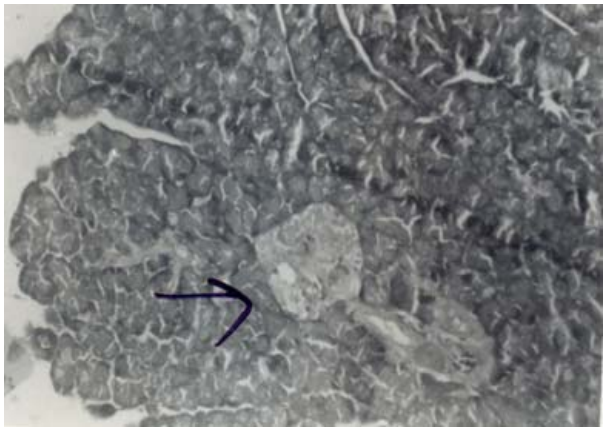

**Fig. 3C.** Pancreas of diabetic rats treated with pterostilbene (10 mg) (H&E x 20). Marked reduction in fatty infiltration of islets (→).

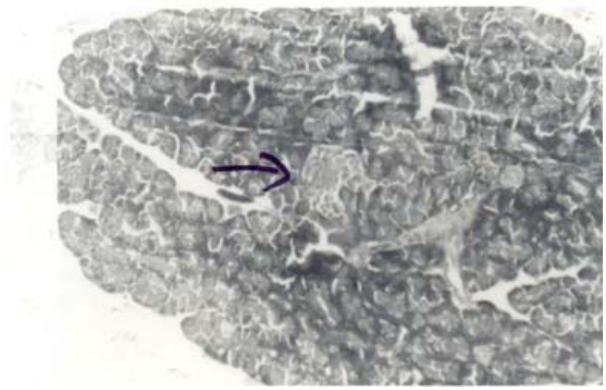

**Fig. 3D.** Pancreas of diabetic rats treated with pterostilbene (20 mg) (H&E x 20). Islet shrinkage (→).

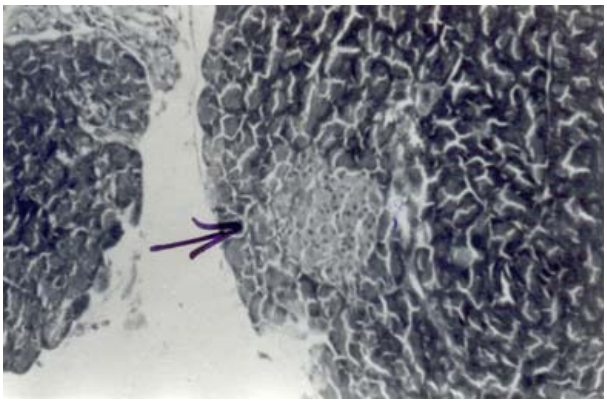

**Fig. 3E.** Pancreas of diabetic rats treated with pterostilbene (40 mg) (H&E x 20). Normal appearance of islets (→).

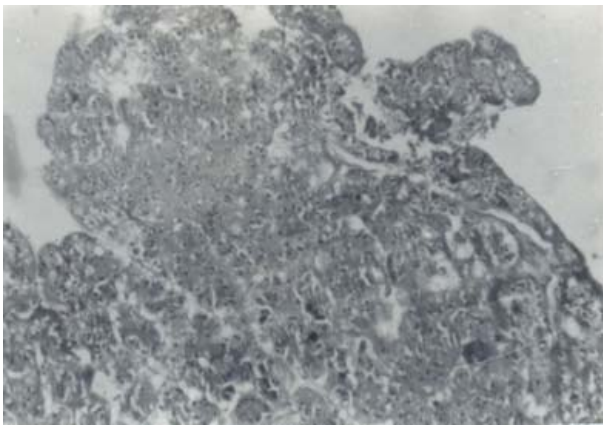

**Fig. 3F.** Pancreas of diabetic rats treated with THC (80 mg) (H&E x 20). Parenchymal inflammation and necrosis.

infiltration of islets and shrinkage (Fig. 3B). Diabetic rats treated with 10, 20 and 40 mg/kg of pterostilbene revealed reduction in fatty infiltration of islets, islet shrinkage and normal appearance of islets (Fig. 3C, D and E), whereas treatment with 80 mg/kg THC showed parenchymal inflammation and necrosis (Fig. 3F). The shrinkage of islets in diabetic control rats and diabetic rats treated with 10, 20 and 40 mg/kg pterostilbene may be due to excessive destruction of islets by STZ that specifically damages islets. In addition, the above doses of pterostilbene were not able to counteract the effect of STZ. Pterostilbene at 0.40 mg/kg induced near to normal appearance of islets, which is probably due to its ability to withstand the detrimental effect of STZ at that concentration and the protection it offers to β-cells leading to increased insulin secretion. Thus in addition to blood glucose lowering effect, histopathological observations also support the notion that pterostilbene at 0.45 mg/kg induces significant anti-hyperglycemic activity by protecting the tissues against STZ action.

## DISCUSSION

Approximately 6.6% of the global population carries out a diagnosis of type 2 diabetes mellitus (T2DM). Patients with T2DM are at greater risk for developing dementia and Alzheimer's disease (AD) and have been reported to exhibit inferior cognitive performance when compared to age-matched healthy controls (HCs) [34]. Several human neuroimaging studies have linked T2DM with brain atrophy and cognition [34]; recent research suggested that T2DM resulted in a more rapid rate of cognitive decline than that typically associated with natural aging [35].

The involvement of free radicals in diabetes and the role of these toxic species in lipid peroxidation and the antioxidant defense system have been studied. For the study of anti-diabetic agents, STZ-induced hyperglycemia in rodents is considered to be a good preliminary screening diabetic model [36] and is widely used. STZ, N-{methylnitrocarbamoyl}-D-glucosamine is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic cells. β-cells are particularly sensitive to damage by nitric oxide and free radical because of their low levels of free radical scavenging enzymes [37]. STZ directly generates oxygen free radical-induced lipid peroxidation. This study was therefore undertaken to assess the anti-peroxidative properties of THC and pterostilbene in STZ diabetic rats. This study showed that 45 days treatment with THC at 80 mg/kg body weight reduces blood glucose levels in STZ-induced diabetic rats. The possible mechanism(s) by which THC initiates anti-hyperglycemic action could be pancreatic secretion of insulin from existing β-cells, enhanced transport of blood glucose to peripheral tissues or reduced glucose absorption from the gastro-intestinal tract. Diabetes also contributes to cerebrovascular complications, reductions in cerebral blood flow, disruption of the blood brain barrier and cerebral edema [22]. All of these neurochemical and neurophysiological changes ultimately contribute to the long-term complications associated with diabetes, including morphological abnormalities, cognitive impairments and increased vulnerability to pathophysiological events [38].

The possible mechanism by which THC brings about its anti-hyperglycemic action may be stimulation of surviving β-cells to release more insulin. This was clearly evidenced by the increased level of insulin in diabetic rats treated with THC.

The administration of THC and pterostilbene to decrease the increased blood glucose concentration to normal glycemic concentration is an essential trigger for the brain to revert its normal homeostasis during experimental diabetes. THC has the ability to trigger the proinsulin synthesis and also insulin release, which might be helpful to reduce the plasma glucose and increase insulin during diabetes.

Studies have reported an increase in brain thiobarbituric acid reactive substances and hydroperoxide concentration in streptozotocin-induced diabetic rats, when compared with the normal rats. In diabetes, hypoinsulinaemia increases the activities of the enzymes fatty acyl coenzyme and coenzyme A oxidase, which initiates β-oxidation of fatty acids resulting in lipid peroxidation [39]. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity, and changing the activity of membrane-bound

enzymes [40]. Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with athrosclerosis and brain damage [40].

This study shows that administration of THC and pterostilbene significantly decreased the brain thiobarbituric acid reactive substances and hydroperoxides. Previous studies reported that THC increased hepatic GSH levels and induced certain forms of GSH transferase important in preventing lipid peroxidation in diabetes [21]. This indicates that inhibition of oxidative damage by THC may be due to its anti-peroxidative effect.

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. Associated with the changes in lipid peroxidation the diabetic tissues showed decreased activities of key antioxidants SOD, CAT, GSH, GPx, GST, GSH, vitamin C and vitamin E, which play an important role in scavenging free radicals. SOD and catalase are the two major scavenging enzymes that remove toxic free radicals in vivo. It is well documented that the activity of SOD is low in diabetes mellitus [41]. Reduced activities of SOD and catalase in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of $O_2^{\bullet -}$ and $H_2O_2$ [20].

Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of oxidative damage. Therefore removing $O_2^{\bullet -}$ and $^{\bullet}OH$ is probably one of the most effective defenses against diseases [42]. Treatment with pterostilbene increased the activities of SOD and catalase in the diabetic rats when compared to diabetic control rats. This clearly shows that pterostilbene possesses free radical scavenging activity, which could exert beneficial action against pathologic alterations caused by $O_2^{\bullet -}$ and $^{\bullet}OH$. Pterostilbene effectively scavenges peroxyl radicals ($ROO^{\bullet}$) and reduces the singlet oxygen-induced peroxidation at levels similar to those of resveratrol [43]. Because of these activities, it was expected that pterostilbene might decrease the utilization of enzymatic antioxidants and reduce the free radical-mediated inactivation of enzyme proteins thereby maintaining the activities of enzymatic antioxidants.

The second line of defense consists of the non-enzymatic scavengers such as GSH which scavenge residual free radicals escaping from decomposition process mediated by the antioxidant enzymes. Moreover, enzymatic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymatic antioxidants is presumably essential for the removal of these radicals [44].

GSH is the most important biomolecule, which participates in the elimination of reactive intermediates by reducing hydroperoxides in the presence of GPx. GSH also functions as a free radical scavenger and plays a role in the repair of radical-caused biological damage [45]. Decreased glutathione levels in type 2 diabetes have been considered as an indicator of increased oxidative stress [46]. The decrease in the GSH level represents the increased utilization in trapping the oxy radicals. GPx and GST catalyse the reduction of $H_2O_2$ and hydroperoxides to non-toxic products [47]. Previous studies reveal that the activities of GPx and GST significantly decreased in diabetic rat tissues [48]. The decreased activities of these enzymes result in deleterious oxidative changes due to the accumulation of toxic products. Administration of pterostilbene and THC increased the content of GSH in the liver of diabetic rats.

## CONCLUSION

In diabetic rats, brain tissue was more vulnerable to oxidative stress and showed increased lipid peroxidation. The above observations showed that THC possesses antioxidant effect that may contribute to its protective action against lipid peroxidation and enhancement of cellular antioxidant defense. This activity contributes to the protection against oxidative damage in STZ-induced diabetes.

## CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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