Original Article

Antioxidant Effect Of Tetrahydrocurcumin Compared Pterostilbene In Streptozotocin - Nicotinamide Induced Diabetic Rats

Pidaran Murugan[1&*]

[1*]Assistant professor of Biochemistry, Centre for Distance and Online Education, Bharathidhasan University, 620024. Tamil Nadu, India.

*Corresponding Author: - Dr. P. Murugan
*Assistant professor of Biochemistry, Centre for Distance Education and Online education, Bharathidhasan University, 620024. Tamil Nadu, India. Email : manomuruganphd@gmail.com Alternative Email: pmpranithmurugan18@gmail.com
DOI: 10.47750/pnr.2022.13.04.295

Abstract

Tetrahydrocurcumin (THC) are polyphenolic compounds with para-hydroxyl functional groups and keto functional groups that participate in antioxidant and chemo preventive action (Sugiyama et al. 1996). THC is a hydrogenation product of curcumin produced by reducing curcumin in an organic solvent using a metal catalyst. Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances. *Pterocarpus marsupium* has been used for many years in the treatment of diabetes mellitus. Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium*. Oxidative stress has been suggested to be a contributory factor in development and complication of diabetes. In the present study, we have investigated the effect of tetrahydrocurcumin (THC), one of the active metabolites of curcumin on antioxidants status in streptozotocin - nicotinamide induced diabetic rats. Oral administration of THC at (80mg/kg body weight) and Pterostilbene (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. In addition, THC and Pterostilbene caused significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, vitamin C and vitamin E in liver and kidney of diabetic rats with significant decrease in thiobarbituric acid reactive substances (TBARS) and hydroperoxides formation in liver and kidney, suggesting its role in protection against lipid peroxidation induced membrane damage. The antidiabetic and antioxidant effects of THC are more potent than those of Pterostilbene.

Keywords: antioxidants, tetrahydrocurcumin, curcumin, lipid peroxidation.

INTRODUCTION

Oxidative stress is defined as the imbalance between free radical production and the antioxidant defense system in favour of the former. Oxidative stress leads alterations in lipid, protein and DNA. Oxidative stress is associated with complications of diabetes and has also been linked to IR (Ceriello, 2000). Very often, oxidative stress is evidenced by assaying the end products of lipid peroxidation in plasma and tissues. Oxidative stress in cells and tissues results from the increased generation of free radicals and/or from decreases in antioxidant defense potential (Frei, 1991). Several mechanisms have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation of glucose, the non-enzymic glycation of proteins with the consequently increased formation of AGEs and enhanced glucose flux through the polyol pathway (Wolf and Dean, 1987; Tesfamariam, 1994; Elgawish et al., 1996).
Accordingly, the current study evaluated the effect of pterostilbene on lipid peroxidation and changes in antioxidant defense in diabetic rats based on analyzing lipid peroxidative markers, free radical scavenging enzymes and non-enzymic antioxidants in plasma, liver and kidney.

THC, produced from curcumin by hydrogenation, are colorless which render these products useful in non-colored food and cosmetic applications that currently employ synthetic antioxidants (Murugan and Pari, 2006). THC is one of the major metabolites of curcumin, with potential bioactivity. This metabolite was identified in intestinal and hepatic cytosol from humans and rats (Murugan and Pari, 2007; Murugan and Pari, 2008). The reduction of curcumin to THC seems to occur primarily in a cytosolic compartment (Ireson, 2002). Final reduction of THC to hexahydrocurcuminol may occur in microsomes (possibly by cytochrome P450 reductase) (Ireson, 2002). Recently, attention has focused on THC, as one of the major metabolites of curcumin, because this compound appears to exert greater antioxidant activity in both in vitro and in vivo systems (Pari and Murugan, 2004). In our previous study, we have demonstrated the antidiabetic effect of THC in streptozotocin (STZ) induced diabetic rats ((Pari and Murugan, 2005).
Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances. Pterocarpus marsupium has been used for many years in the treatment of diabetes mellitus (Warrier, 1995). Pterostilbene was found to be one of the active constituents in the extracts of the heartwood

of Pterocarpus marsupium (Maurya et al. 2004). It is suggested that pterostilbene might be one of the principal anti-diabetic constituents of Pterocarpus marsupium (Maurya et al. 2004). 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).
To our knowledge, so far no other biochemical investigations has been carried out on the effect of THC compared pterostilbene in tissue antioxidant status of experimental diabetic rats. The present investigation was carried out to study the effect of THC compared pterostilbene on tissue lipid peroxides and antioxidants in rats with STZ and nicotinamide induced diabetes.

MATERIALS AND METHODS

Drugs and chemicals

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

Induction of diabetes

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

Experimental design

In the experiment, 24 rats were divided into 4 groups of 6 each, after the induction of STZ diabetes. The experimental period was 45 days.
Group 1: Normal untreated rats. Group 2: Diabetic control rats. Group 3: Diabetic rats given THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Muugan and Pari, 2006). Group 4: Diabetic rats given Pterostilbene (40 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Pari and Amarnath Satheesh, 2006).
At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood.

Preparation of tissue homogenate

The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris - HCl buffer, pH 7.5. After centrifugation at 10,000 X g for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances and hydroperoxides. For the determinations of vitamin E level the liver and kidney tissues were weighed and lipids were extracted from tissues by the method of Folch *et al*. (1957) using chloroform - methanol mixture ($CHCl_3$: MeOH)(2:1; v/v). The extract used for the estimation of vitamin E.
For the estimation of non-enzymic and enzymic antioxidants, tissue was minced and homogenized (10 % w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged for 10 min and the resulting supernatant was used for enzyme assays.

Analytical procedure

Measurement of blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (Lott and Turner, 1975). Plasma insulin was assayed by ELISA 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*. (1988) and Jiang *et al*. (1992), 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, 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/100g-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 (1972). 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*. (1984). 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 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 560nm against butanol blank. A system devoid of enzyme served as control. One unit of the enzyme activity is defined as the enzyme reaction, which gave 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*. (1973). 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*. (1974). 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 is expressed as mM of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6/ mM /cm.

Estimation of ascorbic acid (vitamin C)

Vitamin C was estimated by the method of Omaye *et al*. (1979). 0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6% TCA and centrifuged for 10 min at 3500 X g. After centrifusion, 0.5 ml of the supernatant was mixed with 0.5 ml of DNPH reagent and allowed to stand at room temperature for an additional 3 hours then added 2.5 ml of 85% sulphuric acid and allowed to stand for 30 min. Then the absorbance was read at 530 nm. A set of standards containing 10-50 μg of ascorbic acid were taken and processed similarly along with a blank. Ascorbic acid values were expressed as μM/mg tissue.

Estimation of vitamin E

Vitamin E was determined by the method of Baker *et al*. (1951). 0.1 ml of lipid extract, 1.5 ml of ethanol and 2 ml of petroleum ether were added, mixed and centrifuged for 3000 X g for 10 min. The supernatant was evaporated to dryness at 80°C then 0.2 ml of 2,2l□□□dipyridyl solution and 0.2 ml of ferric chloride solution was added and mixed well. This was kept in dark for 5 min and added 2 ml of butanol. Then the absorbance was read at 520 nm. Standards of α-tocopherol in the range of 10-100 μg were taken and treated similarly along with blank containing only the reagent. The values were expressed as μM/mg – tissue. Protein was determined by the method of Lowry *et al*. (1951).

Estimation of reduced glutathione (GSH)

GSH was determined by the method of Ellman (1959). 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 added to it 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer (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/100g - tissue

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

Changes in blood glucose and plasma insulin

Table 1 shows the level of blood glucose and plasma insulin of different experimental groups. The diabetic control rats showed a significant increase in the level of blood glucose with significant decrease in the activity of plasma insulin.

Oral administration of THC and pterostilbene to diabetic rats significantly reversed the above biochemical changes. The administration of THC and pterostilbene to normal rats showed a significant effect on blood glucose and plasma insulin levels. The THC administration showed more effective than pterostilbene.

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

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (µU/ml)
Normal	95.23 ± 6.35^{a}	12.32 ± 0.73^{a}
Diabetic control	299.35 ± 9.94^{b}	3.87 ± 0.21^{b}
Diabetic + THC (80 mg/kg)	116.54 ± 7.54^{c}	9.54 ± 0.54^{c}
Diabetic + Pterostilbene (40 mg/kg)	139.15 ± 7.12^{d}	8.65 ± 0.47^{d}
Diabetic+ glibenclamide (600 µg/ kg)	139.25 ± 7.65^{d}	8.45 ± 0.39^{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).

Effect of tissue lipid peroxidation

Table 2 represents the concentration of TBARS and hydroperoxides in tissues of normal and experimental rats. There was a significant elevation in tissue TBARS and hydroperoxides during diabetes, when compared to the corresponding normal group. Administration of THC and pterostilbene significantly decreased the lipid peroxidation in diabetic rats. The THC was more potent than pterostilbene.

Table 2. Influence of THC and pterostilbene on the content of TBARS and hydroperoxides in rats liver and kidney.

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Pterostilbene (40 mg/kg)	Diabetic+ Glibencalamide (600 µg/ kg)
TBARS					
Liver (mM/100g tissue)	0.87 ± 0.04^{a}	1.88 ± 0.13^{b}	1.58 ± 0.06^{c}	1.11 ± 0.05^{d}	1.28 ± 0.03 ^{d}
Kidney (mM/100g tissue)	1.78+ ± 0.19^{a}	3.93 ± 0.29^{b}	2.32 ± 0.15^{c}	1.98 ± 0.13^{d}	2.17 ± 0.03^{e}
Hydroperoxides					
Liver (mM/100g tissue)	84.26 ± 5.32^{a}	105.21 ± 5.51^{b}	92.67 ± 4.12^{c}	89.21 ± 4.15^{ac}	87.35 ± 0.04^{d}
Kidney (mM/100g tissue)	58.21 ± 3.15^{a}	82.58 ± 4.48^{b}	69.32 ± 3.54^{c}	64.54 ± 4.24^{c}	73.41 ± 4.38^{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 (Duncan's Multiple Range Test).

Effect on tissue enzymes and antioxidants

For studying the THC and pterostilbene on free radical production, the activities of SOD, CAT, GPx, GST, GSH, vitamin C and vitamin E were measured (table 3 and 4). They presented significant increases in THC and pterostilbene treatment when compared with diabetic control rats. The effect of THC was more prominent compared with pterostilbene.

Table 3. Influence of THC and pterostilbene on the CAT, SOD, GPx, and GST activities in rats liver and kidney

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Pterostilbene (40 mg/kg)	Diabetic+ Glibencalamid e (600 µg/ kg)
CAT (units/mg of protein)					
Liver	77.32 ± 5.54^{a}	48.54 ±3.21^{b}	58.54 ± 3.54^{c}	70.54 ± 4.45^{d}	74.41 ± 4.14^{c}
Kidney	35.21 ± 2.15^{a}	18.27 ± 1.24^{b}	25.21 ± 1.54^{c}	30.54± 1.20^{d}	32.47± 1.40^{c}
SOD (units/mg of protein)					
Liver	7.32 ± 0.21^{a}	3.55 ± 0.24^{b}	4.54 ± 0.14^{c}	5.45 ± 0.34^{d}	6.87 ± 0.32^{c}
Kidney	15.54 ± 0.87^{a}	9.24 ± 0.44^{b}	13.66 ± 0.41^{c}	11.24 ± 0.32^{c}	12.54 ± 0.32^{c}
GPx (units/mg of protein)					
Liver	6.25 ± 0.32^{a}	3.54 ± 0.25^{b}	4.24 ± 0.21^{c}	5.45 ± 0.35^{d}	6.41 ± 0.25^{c}
Kidney	4.21 ± 0.25^{a}	2.45 ± 0.11^{b}	3.58 ± 0.15^{c}	4.54 ± 0.21^{c}	4.54 ± 0.44^{c}
GST (units/mg of protein)					
Liver	6.99 ± 0.54^{a}	3.54 ± 0.25^{b}	5.78 ± 0.48^{c}	5.47 ± 0.35^{d}	6.54 ± 0.45^{c}
Kidney	5.78 ± 0.30^{a}	2.45 ± 0.12^{b}	3.45 ± 0.15^{c}	4.45 ± 0.28^{d}	4.98 ± 0.19^{c}

Data are mean ± SD values for six rats in each group. Units are as follows: CAT, µM of H_2O_2 consumed per minute; SOD, 1 unit of activity equals the enzyme reaction that gave 50% inhibition of nitro blue tetrazolium reduction in 1 minute; GSH, micrograms of GSH consumed per minute; GST, µM of 1-chloro-2, 4-dinitrobenzene-glutathione

(CDNB–GSH) conjugate formed per minute. Values not sharing a common superscript letter differ significantly at $P < .05$ (Duncan's Multiple Range Test).

Table 4. Influence of THC and pterostilbene on content of vitamin C, vitamin E, and GSH in rats liver and kidney

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Pterostilbene (40 mg/kg)	Diabetic+ Glibencalamide (600 µg/ kg)
Vitamin C (µM/mg of tissue)					
Liver	1.50 ± 0.02^{a}	0.85 ± 0.02^{b}	1.15 ± 0.03^{c}	1.35 ± 0.03^{d}	1.40 ± 0.03^{d}
Kidney	1.03 ± 0.04^{a}	0.34 ± 0.02^{b}	0.73 ± 0.02^{c}	0.83 ± 0.03^{d}	0.87 ± 0.01^{d}
Vitamin E (µM/mg of tissue)					
Liver	0.68 ± 0.03^{a}	0.16 ± 0.01^{b}	0.44 ± 0.02^{c}	0.56 ± 0.02^{d}	0.58 ± 0.03^{d}
Kidney	0.47 ± 0.03^{a}	0.09 ± 0.01^{b}	0.32 ± 0.02^{c}	0.38 ± 0.03^{d}	0.39 ± 0.03^{d}
GSH (mg/100 g of tissue)					
Liver	48.21 ± 2.74^{a}	21.55 ± 1.55^{b}	33.77 ± 1.55^{c}	38.54 ± 2.32^{d}	43.24 ± 2.44^{d}
Kidney	36.32 ± 2.15^{a}	19.25 ± 1.28^{b}	25.32 ± 1.24^{c}	32.36 ± 1.45^{d}	33.25 ± 1.62^{d}

Data are mean ± SD values for six rats in each group.
Values not sharing a common superscript letter differ significantly at $P < .05$ (Duncan's Multiple Range Test).

DISCUSSION

Oxidation of PUFAs of membranes (lipid peroxidation) is a common process in living organisms, since they are the target of oxygen derived free radicals produced during mitochondrial electron transport (Porter et al., 1995). Increased lipid peroxidation associated with diabetes, which is an indicator of oxidative stress, has been often determined in both animal models and human clinical trials.

Increase in hydroxyl radical formation in diabetic rats may be elucidated by two biochemical mechanisms. One mechanism is increased production of activated oxygen species such as $O_2^{\bullet -}$ or H_2O_2. $OH^{\bullet}$ radicals are generated from $O_2^{\bullet -}$ or H_2O_2 by the iron catalyzed Haber-weiss reaction or Fenton reaction respectively. Another mechanism is decrease in the activity of enzymes (SOD, CAT and glutathione dependent enzymes) to scavenge the activated oxygen species (Chiou et al. 2003).

Lipid peroxidation is a free-radical mediated propagation of oxidative insult to PUFAs involving several types of free radicals and termination occurs through enzymatic means or by free radical scavenging by antioxidants (Korkina and Afanasev, 1997). In our study, the lipid peroxidation end products measured as TBARS and HP were increased in plasma and tissues of STZ-nicotinamide induced diabetic rats, which might be due to relatively high concentration of easily peroxidizable fatty acids in tissues. During diabetes, liver showed a relatively severe impairment in antioxidant capacity than kidney. The kidney exhibits a characteristic pattern of changes during diabetes (Aragno et al., 1999). The increase of oxygen free radicals in diabetes could be primarily due to increase in blood glucose levels, which upon autoxidation generate free radicals and secondarily due to the effects of diabetogenic agent STZ (Ivorra et al., 1989). Drugs with antioxidant properties may supply endogenous defense systems and reduce both initiation and propagation of ROS (Bergendi, 1999). The antioxidative activity of pterostilbene was first demonstrated *in vitro* by its inhibition of methyl linoleate oxidation (Charvet-Faury et al., 1998). Administration of pterostilbene and THC reduced the lipid peroxidative markers in plasma and tissues of diabetic rats.

Free radical scavenging enzymes such as SOD, catalase and GPx are the first line of defense against oxidative injury. SOD catalyzes to scavenge the excess of superoxide anions and convert them to H_2O_2. The primary role of catalase is to scavenge H_2O_2 that has been generated by free radicals or by SOD in removal of superoxide anions and to convert it in to water. Gpx works in tandem with catalase to scavenge excess of H_2O_2 as well as lipid peroxidation in response to oxidative stress. We have observed a decrease in the activity of SOD and catalase in liver and kidney of diabetic control rats. 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 (Vucic et al., 1997; Feillet-Coudray et al., 1999). 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 (Searle and Wilson, 1981).

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 (Ananthan et al., 2004). Treatment with THC and pterostilbene increased the activities of SOD, catalase in the diabetic rats when compared to diabetic control rats. This clearly shows that THC and pterostilbene possesses free radical scavenging activity, which could exert beneficial action against pathologic alterations caused by the presence of $O_2^{\bullet -}$ and $^{\bullet}OH$. THC and Pterostilbene effectively scavenges peroxyl radicals ($ROO^{\bullet}$) and reduce the singlet-oxygen induced peroxidation at levels similar to those of resveratrol (Rimando et al., 2002). Administration of THC and pterostilbene significantly reversed the changes to near normal levels in the circulation and tissues of diabetic rats. Because of these

activities, it was expected that THC and pterostilbene might decrease the utilization of enzymic antioxidants and reduce the free radical mediated inactivation of enzyme proteins and thereby maintaining the activities of enzymic antioxidants.
The second line of defense consists of the non-enzymic scavengers such as GSH, ascorbic acid and α-tocopherol, which scavenge residual free radicals escaping from decomposition process mediated by the antioxidant enzymes. Moreover, enzymic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymic antioxidants is presumably essential for the removal of these radicals (Allen, 1991).
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 in the repair of radical caused biological damage (Nicotera and Orrenius, 1986). Decreased glutathione levels in type 2 diabetes have been considered to be an indicator of increased oxidative stress (McLennan et al., 1991). 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 (Bruce et al., 1982). Previous studies reported by us as well as by others reveal that the activities of GPx and GST were significantly decreased in diabetic rat tissues. (Dias et al., 2005). The decreased activities of these enzymes result in the involvement of deleterious oxidative changes due to the accumulation of toxic products. Administration of THC and pterostilbene increased the content of GSH in the liver of diabetic rats.
Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to ROS (Frei et al., 1986). Studies involving different types of oxidative stress have shown that under all types of oxidative damage and therefore it would be helpful in prevention of diseases in which oxidative stress plays a causative or exacerbation role. The observed significant decrease in the level of plasma vitamin C could be due to the increased utilisation of vitamin C as an antioxidant defense against ROS or to a decrease in the GSH level, since GSH is required for the recycling of vitamin C.

Vitamin E is an important lipophilic antioxidant, which has an effective role in maintaining the cell structure against oxidative damage through blocking the chain reaction of free radicals. Low levels of plasma antioxidants have been implicated as a risk factor for the development of diabetes (Vatassery et al. 1983). This decrease could have been due to increased utilization of vitamin C as an antioxidant defense against increased ROS or to a decrease in the GSH level, since GSH is required for the recycling of vitamin C. Both vitamin C and vitamin E are known to prevent detectable lipid peroxidation, and under physiological conditions, it has been suggested that vitamin C helps to recycle vitamin E from its radical form (Garg and Bansal, 2000). Oral administration of THC and pterostilbene to diabetic rats restored the level of vitamin E to near normal. It is likely that increased level of vitamin E may be due to increased levels of GSH.

Administration of THC and pterostilbene significantly decreased the levels of lipid peroxidation products in diabetic rats. The antioxidative activity of pterostilbene was first demonstrated in vitro by its inhibition of methyl linoleate oxidation (Charvet-Faury et al., 1998). Recently, pterostilbene was reported to scavenge DPPH free radical and to inhibit the oxidation of citronellal and lipid peroxidation in rat liver microsomes and in cultured human fibroblast (Stivala et al., 2001). Pterostilbene effectively scavenges peroxyl radicals ($ROO^{\bullet}$) and reduce the singlet-oxygen induced peroxidation at levels similar to those of resveratrol.
In conclusion, the present investigation shows that THC possesses antioxidant effect that may contribute to its protective action against lipid peroxidation and enhancing effect on cellular antioxidant defense. This activity contributes to the protection against oxidative damage in STZ induced diabetes. The THC administration showed more effective than pterostilbene and glibenclamide.

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