INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND STUDIES

VOLUME04ISSUE03

'' TETRAHYDROCURCUMIN AND CHLOROGENIC ACID AGAINST STREP TOZOTOCIN–NICOTINAMIDE GENERATED OXIDATIVE STRESS-INDUCED DIABETES''

FASEFAL PLIMAYESHU; KALEA AKILY

ABSTRACT

the present study was undertaken **to analyze** the protective effect of tetra hydrocurcumin (THC) and chlorogenic acid (CGA) against streptozotocin (STZ)–nico-tinamide (NA)-induced type 2 diabetes in adult Wistar rats. Diabetes was induced in experimental rats weighing 180–220 g, by **one** intraperitoneal (i.p.) injection of STZ (45 mg/kg BW), 15 min after the (i.p.) administration of NA (110 mg/kg BW). THC (80 mg/kg BW) and CGA (5 mg/kg BW) were orally administered to diabetic rats for a period of 45 days. Fasting plasma glucose, glycosylated haemoglobin (HbA1C), thiobarbituric acid reactive substances (TBARS), and lipid hydroperoxides (LOOH) were significantly increased, whereas insulin, total haemoglobin (Hb), non-enzymic antioxidants (reduced glutathione (GSH), vitamin C, vitamin E, and ceruloplasmin) were decreased significantly in diabetic rats. Though the diabetic rats treated with THC and CGA individual exerts beneficial effects **altogether** the biochemical parameters in (STZ)-induced diabetic rats. The combined treatment with THC and CGA normalized all the above-mentioned biochemical parameters in STZ-induced diabetic rats. Normal pancreatic histological architecture in THC and CGA-treated diabetic rats revealed that these phytochemicals exert **a better** degree of protection when administered **together** than **one** treatment of individual compounds.

KEYWORDS

Antioxidants Chlorogenic acid Streptozotocin Tetra-hydrocurcumin,

1. INTRODUCTION

Diabetes mellitus **could be a** chronic disease caused by an inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secrete

insulin (Matsui et al., 2007). Type 2 **DM** (T2-DM) is **one amongst the fore-most** common metabolic illnesses worldwide. **within the** year 2000 diabetes prevalence was estimated in 180 million people worldwide and this number **is predicted** to double in 2030 (WHO, 2008). Currently, oral hypoglycemic drugs like sulfonylurea derivatives, biguanides, and thiazolidinediones, or injectable insulin, are used for treating T2-DM. However, **of these** agents display undesirable side effects, and, ultimately, all of them fail **to revive** glycemic control (Spiller & Sawyer, 2006). Thus, **it's** essential **to seem** for **more practical** antidiabetic agents preferably from dietary sources, which should be economical and non-toxic or less toxic.

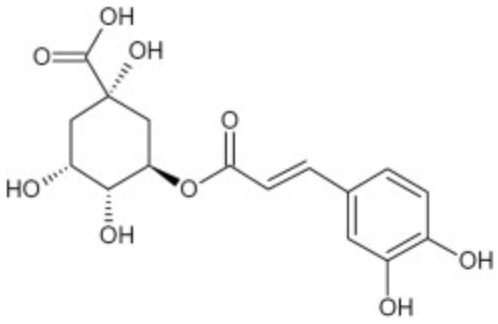
Streptozotocin (STZ), an antibiotic produced by Streptomyces chromogens, has been widely used for inducing diabetes in experimental animals through its toxic effects on pancreatic β-cells (Kim et al., 2003). The cytotoxic action of STZ is **related to** the generation of reactive oxygen species (ROS) causing oxidative damage that culminates in β-cell destruction through the induction of apoptosis and suppression of insulin biosynthesis (Szkudelski, 2001). Diabetics and experimental animal models were shown to a degree of exhibit high oxidative stress **because of** persistent and chronic hyperglycemia, which thereby depletes the activity of the antioxidative **defence system** and thus promotes de novo free radicals generation (Bayness & Thorpe, 1996). Chemicals with antioxidant properties and **atom** scavengers were shown **to stop** pancreatic islets against cytotoxic effects of STZ (Coskun et al., 2005).

Oxidative stress depicts the existence of products called free radicals and ROS, which are formed under normal physiological conditions but become deleterious when not being quenched by the antioxidant systems (Fang et al., 2002). **there's** convincing experimental and clinical evidence that the generation of reactive oxygen species is increased in both **the kind** of diabetes **which** the onset of diabetes is closely **related to** oxidative stress (Rosen et al., 2001, Johansen et al., 2005). Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway, and non-enzymic glycation of proteins (Wolff and Dean, 1987, Brownlee et al., 1988, Obrosova

et al., 2002). As a consequence of hyperglycemia abnormally high levels of free radicals, decline of antioxidant defense systems (Maritim et al., 2003, Seif and Youssef, 2004), lowered vitamin levels (West, 2000); and decreased ceruloplasmin levels have also been reported (Anwar and Meki, 2003, Seif and Youssef, 2004). It can **cause** damage of cellular organelles and enzymes increase lipid peroxidation (LPO) and development of complications of **DM** (Maritim et al., 2003). Diminished LPO and enhanced antioxidant status **is also** one mechanism by which dietary treatment contributes to the prevention of diabetic complications (Armstrong et al., 1996).

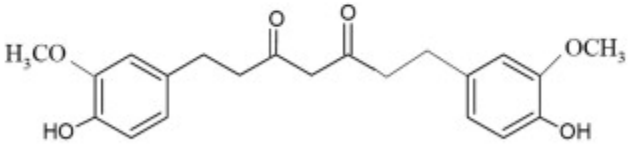
Antioxidant phytochemicals from edible and medicinal plants **are** proposed **because the** major dietary antioxidants providing health benefits (Kasai et al., 2000, Alonso et al., 2006, Rangkadilok et al., 2007). Chlorogenic acid (CGA) is an ester of caffeic with quinic acids (Fig. 1). CGA **may be a** catholic acid **that's** found in almost every plant **and occasional** drink (Buren et al., 1973). The biological and pharmacological properties of CGA are primary attributed to its capacity to donate hydrogen atoms of the phenolic ring to free radicals, thus inhibiting the oxidation process (Zang et al., 2003). tetrahydrocurcumin (THC) **may be a** polyphenolic compound, formed during the metabolism of curcumin (Fig. 2). It occurs primarily in turmeric, which is widely used as a spice and a coloring agent in several foods **like** curry, mustard, and potato chips **furthermore** as in cosmetics **and medicines** (Joe et al., 2004; Okada et al., 2001). THC **incorporates a** high antioxidant potential **thanks to** its resonance-stabilized hydroxy radical structure. **it's an efficient** scavenger of free radicals and has been approved in certain countries as a **additive to stop** LPO (Priyadarsini et al., 2003). Murugan and Pari (2006) have reported that THC stimulates insulin secretion in rat pancreatic β-cells. THC also exhibits strong anti-inflammatory properties (Nakamura, 1998, Hong et al., 2004), anti-atherosclerotic (Naito et al., 2002), hepatoprotective (Pari & Murugan, 2004), and

nephroprotective (Pari & Murugan, 2006).



Download : Download full-size image

Fig. 1. Chemical structure of chlorogenic acid.



Download : Download full-size image

Fig. 2. Chemical structure of tetrahydrocurcumin.

.

According to Medical Practitioners, **a mix of medicine** exhibit augmented protective efficacy than **one** drug. Recently, we reported that combined treatment of THC and CGA exhibits better antidiabetic property with beneficial effects on glycoprotein metabolism in STZ-nicotinamide (NA)-induced diabetic rats (Pari and Karthikesan, 2009, Karthikesan et al., 2010). **within the** present study, we evaluated plasma glucose, insulin, total hemoglobin (Hb), glycosylated hemoglobin (HbA1C), LPO products, and antioxidants **within the** plasma of STZ-NA-induced type 2 diabetic rats. Besides, **the consequences** of THC and CGA on the histopathological alterations of the pancreas in nor-

mal and diabetic rats were also studied.

2. Materials and methods

2.1. Animals

The whole experiment was **dispensed in line with the rules** of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Animal Ethical Committee of Annamalai University (Approval No. 458, 2007). The study was conducted on thirty male albino Wistar rats (Rattus norvegicus) weighing 180–220 g, obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages (47 × 34 × 20 cm), (six rats per cage) lined with husk, renewed every 24 h under a 12:12 h light-dark cycle at around 22 °C, and had free access to **H2O** and pellet diet (Pranav Agro Industries Limited, Maharashtra, India). The pellet diet consists **of twenty-two**.02% protein, 4.25% fat, 3.02% fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% carbohydrates. It provided metabolizable energy of 3600 kcal/kg.

2.2. Chemicals

Chlorogenic acid (CGA) and streptozotocin (STZ) were obtained from Sigma Chemical Co (St. Louis, MO, USA). tetrahydrocurcumin (THC) was **a present** sample from the Sabinsa Corporation, Louis, USA. Nicotinamide (NA) was procured from Ranbaxy Chemicals Ltd., Mumbai, India. All other reagents **employed in** the experiments were of analytical grade **and therefore the** highest purity.

2.3. Induction of type 2 DM

After a 12 h fast, animals **got one** intraperitoneal (i.p.) injection of STZ (45 mg/kg BW in 0.1 M citrate buffer, pH 4.5), 15 min after the i.p. administration of NA (110 mg/kg BW in normal physiological saline) (Masiello et al., 1998). Also, **an answer** of 20% glucose is provided **to stop** hypoglycemia. After 72 h, plasma glucose **was resolute and peo-**

ple rats with fasting glucose levels greater than 250 mg/dL were **utilized in this** study.

2.4. Experimental timeline

The rats were divided into five groups of six animals and had free access to food and water and were treated for 45 days as follows: Group I: normal rats, Group II: diabetic control rats, Group III: CGA (5 mg/kg/day p.o) treated diabetic rats, Group IV: THC (80 mg/kg/day p.o) treated diabetic rats, Group V: CGA and THC (5 + 80 mg/kg/day p.o) treated diabetic rats.

After the last treatment, the rats fasted for 16 h. the animals were then sacrificed by cervical decapitation, and blood samples were collected **within the** test tubes containing potassium oxalate and **salt** (3:1) as an anticoagulant for the estimation of plasma glucose, insulin, lipid peroxidation, and non-enzymic antioxidants. Hemoglobin and glycosylated hemoglobin were estimated in **blood** samples.

2.5. Biochemical analysis

2.5.1. Measurement of plasma glucose, insulin, hemoglobin (Hb), and glycosylated hemoglobin (HbA1C)

Plasma glucose levels were estimated **employing a** commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) by **the tactic** of Trinder (1969). Plasma insulin was assayed by ELISA kit (Boeheringer–Manneheim Kit, Manneheim, Germany). Hb and HbA1C were estimated by Diagnostic kit-Bio Systems (Costa Brava, Spain).

2.5.2. Estimation of lipid peroxidation (LPO)

LPO in plasma was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) by **the strategy** of Niehius and Samuelsson (1968) and Jiang et al. (1992), respectively. In brief, plasma (0.1 mL) was treated with 2 mL of thiobarbituric acid (TBA)–trichloroacetic acid (TCA)–hydrochloric acid (HCl) reagent (0.37% TBA, 0.25 M HCl and 15% TCA, 1:1:1 ratio) placed **in an exceedingly** water bath for 15 min and cooled and centrifuged at room

temperature; clear supernatant was measured at 535 nm against a reagent blank.

A 0.1 mL aliquot of plasma was treated with 0.9 mL of Fox reagent (88 mg of butylated hydroxytoluene, 7.6 mg of xylenol orange, and 0.8 mg of ammonium iron sulfate were added to 90 mL methanol and 10 mL of 250 mM sulphuric acid) and incubated at 37 °C for 30 min. **the colour** that developed was read at 560 nm.

2.5.3. Determination of plasma antioxidants

Reduced glutathione was estimated by **the tactic** of Ellman (1959). A 0.1 mL of plasma was precipitated with 5% TCA. The contents were mixed well for complete precipitation of proteins and centrifuged. To an aliquot of clear supernatant, 2.0 mL of 0.6 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) reagent and 0.2 M phosphate buffer (pH 8.6) were added **to get** a final volume of 4.0 mL. The absorbance was read at 412 nm against a blank containing 5% TCA **rather than** a sample. A series of standards treated similarly were also run **to work out** the glutathione content. **the number** of glutathione was expressed as mg/dL in plasma.

Vitamin E (α-tocopherol) was estimated by **the tactic** of Desai (1984). To 1.0 mL of plasma 1.0 mL of ethanol was added and thoroughly mixed. Then 3 mL of petroleum ether was added, shaken rapidly, and centrifuged (600g). Two milliliters of supernatant were taken and evaporated to dryness. To this 0.2 mL of 0.2%, bathophenanthroline was added. The assay mixture was **shielded from** light and 0.2 mL of 0.001 M ferric chloride was added followed by 0.2 mL of 0.001 M o-phosphoric acid. **the whole** volume was made up **to three** mL with ethanol. **the colour** developed was read at 530 nm. **the extent** of **E** was expressed as mg/dL of plasma.

Ascorbic acid (Vitamin C) concentration was measured by **the strategy** of Omaye et al. (1979). To 0.5 mL of plasma, 1.5 mL of 6% TCA was added and centrifuged (3500g, 20 min). To 0.5 mL of supernatant, 0.5 mL of dinitrophenylhydrazine (DNPH) reagent (2%

DNPH and 4% thiourea in 4.5 M sulphuric acid) was added, and incubated for 3 h at **temperature**.

Plasma ceruloplasmin level was estimated by **the tactic** of Ravin (1961). To 8.0 mL of acetate buffer (0.4 M, pH 5.5), added 0.05 mL of plasma, 1.0 mL of 0.5% p-phenylenediamine hydrochloride, mixed and kept at 37 °C for 1 h. One mL of 0.5% sodium azide was added and kept at 4 °C for 30 min. **a sway** tube was carried **together with** the test where sodium azide was added before the addition of p-phenylenediamine hydrochloride. **the colour** developed was read at 540 nm with control as blank.

2.6. Histopathological procedures

Pancreatic tissues were harvested from the sacrificed animals, **and also the** fragments from tissues were fixed in 10% neutral formalin solution, embedded in paraffin **then**, stained with hematoxylin and eosin (H&E).

2.7. Statistical analysis of the info

The experimental results are expressed **because the** means ± S.D. and were subjected to one-way analysis of variance (ANOVA), **employing a** computer software package (SPSS version 16.0, SPSS Inc, Cary, NC), **and therefore the** comparisons **of great** groups were performed using the Duncan Multiple Range Test (DMRT) (Duncan, 1957) at $p < 0.05$.

3. Results

3.1. Plasma glucose and insulin

Table 1 showed **the amount** of plasma glucose and insulin of normal and experimental animals. There was **a major** ($p < 0.05$) elevation in plasma glucose, while **the degree** of insulin significantly ($p < 0.05$) decreased **within the** diabetic control animals as compared with **the traditional** group. **a big** ($p < 0.05$) decrease in plasma glucose and increase in insulin levels were observed in diabetic rats treated with CGA or THC. On **the opposite** hand, the effect of **the mixture** of CGA and THC brought plasma glucose and insulin towards near normal values, while normal rats **didn't** exhibit any significant al-

terations in plasma glucose and insulin levels duration of the experimental period.

Table 1. Changes **within the** levels of plasma glucose, insulin, hemoglobin (Hb), and glycosylated hemoglobin (HbA1C) in normal and experimental rats.

Groups Glucose (mg/dL) Insulin (μU/mL) Hb (g/dL) HbA1C (mg/g Hb)
Normal 94.02 ± 7.72a 13.02 ± 1.03a 12.09 ± 0.93a 0.26 ± 0.02a
Diabetic control 284.12 ± 9.09b 3.94 ± 0.04b 9.20 ± 0.70b 0.70 ± 0.05b
Diabetic + THC (80 mg/kg) 110.42 ± 7.56c 9.48 ± 0.44c 10.98 ± 0.85c 0.35 ± 0.03c
Diabetic + CGA (5 mg/kg) 109.48 ± 8.25c 9.34 ± 0.07c 10.89 ± 0.84c 0.42 ± 0.03d
Diabetic + THC/CGA (80 + 5 mg/kg) 95.20 ± 8.45a 12.96 ± 1.13a 12.06 ± 0.93a 0.24 ± 0.02a
Each value is mean ± S.D. **for six** rats in each group.

a–d
In each column, means with different superscript letters differ significantly at $p < 0.05$ (DMRT). THC: tetrahydrocurcumin; CGA: chlorogenic acid.

3.2. Haemoglobin (Hb) and glycosylated hemoglobin (HbA1C)

Significantly ($p < 0.05$) decreased levels of total Hb and increased levels of HbA1C were observed in diabetic control rats **when put next** with normal rats (Table 1). On oral administration of CGA and THC to diabetic rats significantly ($p < 0.05$) increased total Hb and decreased HbA1C **in comparison** with individual treatment to diabetic control rats.

3.3. Lipid peroxidation (LPO)

STZ-treated rats showed **a major** ($p < 0.05$) increase **within the** levels of plasma TBARS and LOOH **compared** to normal rats. Combined treatment with THC and CGA normalized **the degree** of TBARS and LOOH in STZ-treated rats (Fig. 3, Fig. 4).

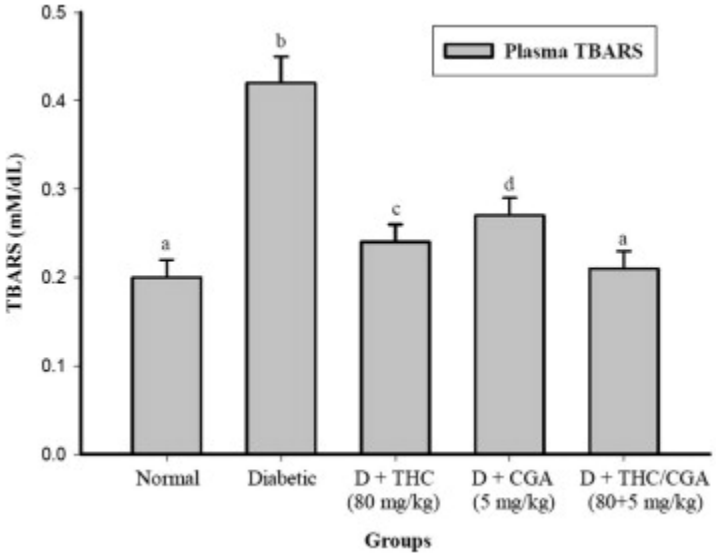


Fig. 3. Effects of tetrahydrocurcumin (THC) and chlorogenic acid (CGA) on plasma TBARS in normal and diabetic rats. a–dIn each bar, means with different superscript letters differ significantly at $p < 0.05$ (DMRT).

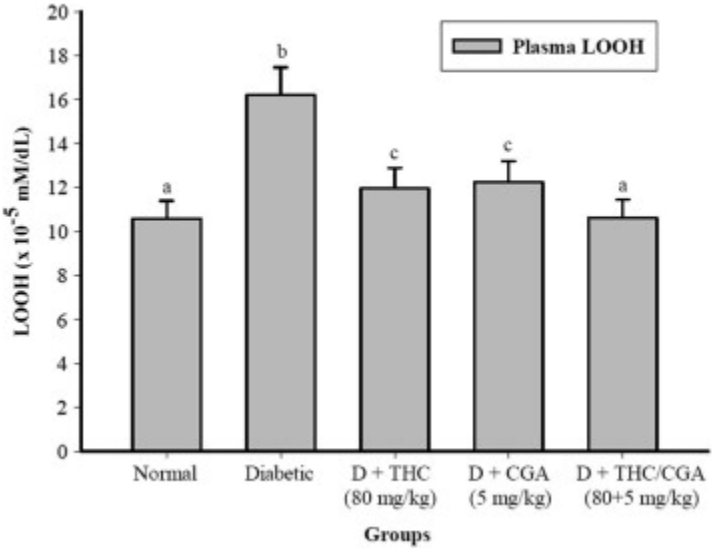


Fig. 4. Effects of tetrahydrocurcumin (THC) and chlorogenic acid (CGA) on plasma

LOOH in normal and diabetic rats. a–can each bar, means with different superscript letters differ significantly at $p < 0.05$ (DMRT).

3.4. Non-enzymic antioxidants

The levels of non-enzymic antioxidants in normal and diabetic rats are given in Table 2. There was **a major** ($p < 0.05$) decrease **within the** levels of reduced glutathione (GSH), vitamin C, vitamin E, and ceruloplasmin in diabetic control rats than normal rats. Combined oral administration of CGA and THC to diabetic rats **ends up in a big** ($p < 0.05$) increase **within the** plasma levels of GSH, vitamin C, vitamin E, and ceruloplasmin.

Table 2. Combined effects of tetrahydrocurcumin (THC) and chlorogenic acid (CGA) on plasma **antioxidant**, vitamin E, reduced glutathione, and ceruloplasmin in normal and experimental rats.

Groups Vitamin C (mg/dL) Vitamin E (α-tocopherol) (mg/dL) Reduced glutathione (mg/dL) Ceruloplasmin (nmol/L)
Normal 1.76 ± 0.13a 1.78 ± 0.14a 24.86 ± 1.90a 1.36 ± 0.11a
Diabetic control 0.70 ± 0.05b 0.58 ± 0.04b 11.83 ± 0.92b 0.86 ± 0.06b
Diabetic + THC (80 mg/kg) 1.52 ± 0.12c 1.60 ± 0.12c 18.54 ± 1.41c 1.22 ± 0.10c
Diabetic + CGA (5 mg/kg) 1.54 ± 0.12c 1.56 ± 0.12c 19.28 ± 1.49c 1.20 ± 0.08c
Diabetic + THC/CGA (80 + 5 mg/kg) 1.74 ± 0.13a 1.76 ± 0.13a 22.58 ± 1.73d 1.33 ± 0.10a
Each value is mean ± S.D. **for six** rats in each group.
a–d
In each column, means with different superscript letters differ significantly at $p < 0.05$ (DMRT).

3.5. Histological results

Based on H and E stained tissue sections (Fig. 5), **there have been** no notable changes in pancreas histology in normal rats (a). In contrast, STZ and NA administration re-

sulted in marked microvesicular and macrovesicular changes in diabetic rats (b). **one** treatment with CGA (5 mg/kg) showed **a light** increase **within the** cellularity of β-cell islets of the pancreas **compared** to diabetic rats (c). Diabetic rats treated with THC (80 mg/kg) showed **a decent** increase **within the** cellularity of β-cell islets of the pancreas **when put next** to diabetic rats (d). Diabetic rats showed cellularity of pancreatic β-cell islets restored **to close** normal upon treatment with CGA (5 mg/kg) and THC (80 mg/kg) (e).

Download: Download full-size image

Fig. 5. Histology of pancreas on treatment with CGA and THC, STZ and NA; H and E staining 400×. (A) Normal: shows normal pancreatic β-cell islets with normal cellularity and normal histology of the pancreas. (B) Diabetic control: shows marked microvesicular and macrovesicular changes of the pancreas. (C) Diabetic control + CGA shows **a light** increase **within the** cellularity of β-cell islets of the pancreas **in comparison** to the STZ-induced pancreas. (D) Diabetic control + THC: This shows **a decent** increase **within the** cellularity of β-cell islets of the pancreas **when put next** to the STZ-induced pancreas. (E) Diabetic control + CGA + THC: shows **close to** normal cellularity of pancreatic β-cell islets **in comparison** to normal pancreas.

4. Discussion

Diabetic experimental animal models exhibit high oxidative stress **thanks to** persistent and chronic hyperglycemia, which depletes the activity of the antioxidative **weaponry leading to** elevated levels of oxygen free radicals (Ihara et al., 1999). Consequences of oxidative stress induce **the assembly** of highly reactive oxygen radicals that are toxic to cells, particularly the **semipermeable membrane during which** these radicals interact with the lipid bilayer and produce lipid peroxides and **result in** pancreatic β-cell dysfunction **yet** as other cellular organelles damage (Haugaard, 1968). Reduced oxidative stress **within the** diabetic condition had been observed in experimental animals following the administration of certain polyphenols

(Sanders et al., 2001).

In the present study, STZ-administered rats showed increased plasma glucose and decreased insulin levels. Combined administration of THC and CGA to diabetic rats showed **the degree** of plasma glucose and insulin towards near normalcy **in comparison** with their individual effects. THC and CGA by their ability to scavenge free radicals and to inhibit lipid peroxidation prevents STZ-induced oxidative stress and protects β-cells, **leading to** increased insulin secretion and decreased plasma glucose levels. **during this** context, recent research by Murugan and Pari (2006) has shown that THC decreased **glucose** concentration and increased insulin release in STZ-induced diabetic rats. THC protected pancreatic β-cells by decreasing oxidative stress and preserving pancreatic β-cell integrity. Besides, CGA **could be a** novel insulin sensitizer that potentiates insulin action **kind of like** the therapeutic action of metformin (McCarty, 2005). CGA reduces **blood sugar** level under its ability to inhibit glucose-6-phosphatase activity, with the related effects on hepatic glycogenolysis (Parker et al., 1998).

Persistent hyperglycemia **ends up in** the glycation of Hb that **results in** the formation of HbA1C (Yabe-Nishimura, 1998). The observed increase **within the** levels of HbA1C with a concomitant decrease in Hb **within the** experimental diabetic rats implies the oxidation of sugars, extensive damage to both sugars and proteins **within the** circulation, vascular wall, and lens proteins thereby continuing and reinforcing the cycle of oxidative stress and damage (Huebschmann et al., 2006). Agents with antioxidant or **atom** scavenging power **are** shown to inhibit oxidative reactions **related to** glycation (Elgawish et al., 1996). **during this** regard combined treatment with THC and CGA significantly reversed the oxidative stress associated/induced changes, which **may be because of** an improvement in insulin secretion.

It is **well-known** that hyperglycemia increases LPO, **which can** contribute to free radical-induced processes **resulting in** the oxidative deterioration of polyunsaturated fatty

acids. Karpen et al. (1982) observed an elevated level of lipid peroxides **within the** plasma of diabetic rats and lipid peroxidation is **one in all** the characteristic features of chronic diabetes. **within the** present study LPO end products measured as TBARS and LOOH were found to be increased **within the** plasma of STZ-diabetic rats. Drugs with antioxidant properties **could provide** endogenous defense systems and reduce both initiation and propagation of reactive oxygen species (Bergendi et al., 1999). Combined treatment with THC and CGA significantly decreased the LPO, through **atom** scavenging activities. Further, the curcuminoids including curcumin and dihydro curcumin **are** reported **for his or her** non-enzymic antioxidant action, which **is principally** from their reducing power and interactions with biological membranes and/or other antioxidant agents (Priyadarsini, 1997, Anusuya and Menon, 2003). **during this** context, THC and CGA could **even have** non-enzymic antioxidant actions **which may** be **chargeable for** their ability to inhibit the LPO (Khopde et al., 2000, Murugan and Pari, 2006, Xiang and Ning, 2008, Sasaki et al., 2010).

Apart from the enzymic antioxidants, non-enzymic antioxidants **like** GSH, vitamin C, vitamin E, and ceruloplasmin play **a superb** role in preventing the cells from oxidative threats. GSH is an intracellular thiol-rich tripeptide, which plays **a significant** role **within the** protection of cells and tissue structures (Yu, 1994). GSH is required for the recycling of **ascorbic acid** (Halliwell and Gutterridge, 1994, Hunt, 1996) and acts as a substrate for GPx and GST that are involved in preventing the deleterious effect of oxygen radicals (Levine, 1990). In our study, diabetic rats exhibited a decreased level of GSH, **which could result** to increased utilization of GSH for scavenging free radicals by GPx and GST. Co-administration of THC and CGA reversed GSH levels **within the** plasma of diabetic rats, which **can be thanks to** the low peroxidisability and thus its low utilization.

Vitamin E **may be a well-known** physiological antioxidant and membrane stabilizer. It interrupts the chain reaction of LPO by reacting with lipid peroxy radicals, thus protecting the cell structures against damage (Ingold et al., 1987). The decreased level

of **vitamin E** observed **within the** diabetic rats is compatible with the hypothesis that the plasma **tocopherol** plays a protective role against increased peroxidation in diabetes. **water-soluble vitamin may be a** hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species. The observed significant decrease **within the** level of plasma **water-soluble vitamin can be** caused by increased utilization of **vitamin C** as an antioxidant defense against ROS or by a decrease in GSH, which is required for the recycling of **ascorbic acid**. Treatment with THC and CGA brought **vitamin C** and E **to close** normal levels which **can be** as a **results of** decreased membrane damage as evidenced by the antioxidant nature. The plasma ceruloplasmin **could be a** powerful **atom** scavenger that oxidizes iron from the ferrous to ferric state.

Protection of β-cells against chronic hyperglycemia-induced damage is **a crucial** target for the treatment of T2-DM (Jin et al., 2008). STZ **is understood** to destroy insulin-producing pancreatic β-cells **and therefore the** STZ-treated rat model **would seem** to represent **a decent** experimental type 2 diabetic state, with residual or remnant insulin production by the pancreatic β-cells (Ohkuwa et al., 1995). Chemicals with antioxidant properties and **radical** scavengers were shown **to forestall** pancreatic islets against cytotoxic effects of STZ (Alvarez et al., 2004). Hence, the pancreatic tissue sections were subjected to H and E staining to picture the histological alterations induced by STZ and to depict the protective effect of THC and CGA on **the identical**. The diabetic pancreas showed marked microvesicular and macrovesicular changes. THC and CGA alone treated diabetic pancreas showed **a light** and good increase **within the** cellularity of β-cell islets of Langerhans. Besides, combined administration of THC and CGA showed near-normal cellularity of pancreatic β-cell islets **within the** diabetic pancreas **when put next** with normal pancreas. This reflects that THC and CGA restore pancreas histology by alleviating oxidative stress induced by STZ. Further, also THC and CGA increases **the scale** of the islets by their ability to regenerate β-cells.

The dietary polyphenolic compounds of THC and CGA possess suitable pharmacokinet-

ic properties. Orally administrated CGA reaches the colon, were hydrolyzed by the colonic microflora converted into caffeic and quinic acids (Plumb et al., 1999, Olthof et al., 2001). Then, the quinic acid moiety is dehydroxylated into cyclohexane **acid and so** aromatized into **carboxylic acid** by the colonic microflora (Cotran et al., 1960, Adamson et al., 1969). The **carboxylic acid** formed is conjugated with glycine and excreted in urine as hippuric acid. Nardini et al. and Olthof et al. determined high levels of conjugated caffeic acid in human plasma, but no CGA, after consumption of coffee and apple cider, respectively (Olthof et al., 2001, Nardini et al., 2002). These results were in good agreement with previous studies in rats, which demonstrated that after ingestion of CGA, conjugated caffeic and ferulic acids, but not intact CGA, were found in plasma and urine (Booth et al., 1957, Choudhury et al., 1999, Azuma et al., 2000). Besides, curcumin (0.1 g/kg) administered intraperitoneally to the mouse was found to undergo metabolic reduction to (occur primarily **in an exceedingly** cytosolic compartment **within the** intestinal and/or hepatic, possibly via a reductase enzyme) dihydro curcumin and tetrahydrocurcumin, which, in turn, were converted to monoglucuronide and sulfate conjugate, which are excreted primarily in bile and to a lesser extent **within the** urine (Pan et al., 1999).

Several studies specify that dietary supplementation with antioxidant nutrients **could also be a secure and easy** complement to traditional therapies for preventing and treating diabetic complications (Ruhe & McDonald, 2001). **the 2** promising phytochemicals **like** THC and CGA are having **an honest** antioxidant property, as evidenced by increased antioxidant status and decreased lipid peroxidation, **which can** protect from **the chance** of diabetic complications. From the results, the combined dose of THC and CGA to diabetic rats showed maximum protective effect compared to individual treatments.

REFERENCES

Adamson et al., 1969
R.H. Adamson, J.W. Bridges, M.E. Evans, R.T. Williams

The role of gut bacteria **within the** aromatization of quinic acid **in several** species
Biochemical Journal, 112 (1969), p. 17P
Google Scholar

Alonso et al., 2006
G.J. Alonso, G.J. Ros, M. Periago
Antiproliferative and cytoprotective activities of a phenolic-rich juice in HepG2 cells
Food Research International, 39 (2006), pp. 982-991
Google Scholar

Alvarez et al., 2004
J.F. Alvarez, A. Barbera, B. Nadal, S. Barcelo-Batllori, S. Piquer, M. Claret, et al.
Stable and functional regeneration of pancreatic beta-cell population in n-STZ rats treated with tungstate
Diabetologia, 47 (2004), pp. 470-477
Google Scholar

Anusuya and Menon, 2003
S. Anusuya, V.P. Menon
Protection of pancreatic β-cell by the potential antioxidant bis-o-hydroxycinnamoyl methane, analog of natural curcuminoid in experimental diabetes
Journal of Pharmacology and Pharmaceutical Science, 6 (2003), pp. 327-333
Google Scholar

Anwar and Meki, 2003
M.M. Anwar, A.R.M.A. Meki
Oxidative stress in streptozotocin-induced diabetic rats: Effects of garlic oil and melatonin Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology, 135 (2003), pp. 539-547

ArticleDownload PDFView Record in ScopusGoogle Scholar

Armstrong et al., 1996
A.J. Armstrong, M. Chestnutt, Gormley, I. Young
The effect of dietary treatment on lipid peroxidation and antioxidant status in newly di-agnosed **adult-onset diabetes mell**
Free Radical Biology and Medicine, 21 (1996), pp. 719-726
ArticleDownload PDFView Record in ScopusGoogle Scholar

Azuma et al., 2000
K. Azuma, K. Ippoushi, M. Nakayama, H. Ito, H. Higashio, J. Terao
Absorption of chlorogenic acid and caffeic acid in rats after oral administration
Journal of Agricultural and Food Chemistry, 48 (2000), pp. 5496-5500
View Record in Scopus Google Scholar

Bayless and Thorpe, 1996
J.W. Baynes, S.R. Thorpe
The role of oxidative stress in diabetic complications
Current Opinion in Endocrinology, 3 (1996), pp. 277-284
Google Scholar

Bergen et al., 1999
L. Bergendi, L. Benes, Z. Durackova, M. Ferencik
Chemistry, physiology, and pathology of free radicals
Life Sciences, 65 (1999), pp. 1865-1874
ArticleDownload PDFView Record in ScopusGoogle Scholar

Booth et al., 1957
A.N. Booth, O.H. Emerson, F.T. Jones, F. De Eds
Urinary metabolites of caffeic and chlorogenic acids

Journal of Biological Chemistry, 229 (1957), pp. 51-59
ArticleDownload PDFView Record in ScopusGoogle Scholar

Brownlee et al., 1988
M. Brownlee, A. Cerami, H. Vlassara
Advanced glycosylation end products in tissue **and therefore the** biochemical basis of diabetic complications
New England Journal **of drugs**, 318 (1988), pp. 1315-1321
Google Scholar

Buren et al., 1973
J.V. Buren, L.D. Vos, W. Pilnik
Measurement of chlorogenic acid and flavonol glycosides in **fruit crush** by a chromatographic–fluorometric method
Journal of Food Science, 38 (1973), pp. 656-658
CrossRefView Record in ScopusGoogle Scholar

Choudhury et al., 1999
R. Choudhury, S.K. Srai, E. Debnam, C.A. Rice-Evans
Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration Free Radical Biology and Medicine, 27 (1999), pp. 278-286
ArticleDownload PDFView Record in ScopusGoogle Scholar

Coskun et al., 2005
O. Coskun, M. Kanter, A. Korkmaz, S. Oter
Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas
Pharmacological Research, 51 (2005), pp. 117-123
ArticleDownload PDFView Record in ScopusGoogle Scholar

Cothran et al., 1960
R. Cothran, M.I. Kendrick, E.H. Kass
Role of intestinal bacteria in aromatization of quinic acid in man and guinea pig
Proceedings of the Society for Experimental Biology and Medicine, 104 (1960), pp. 424-426 CrossRefView Record in ScopusGoogle Scholar

Desai, 1984
I.D. Desai
Vitamin E analysis method for animal tissues
Methods in Enzymology, 105 (1984), pp. 138-143
View Record in ScopusGoogle Scholar

Dormandy, 1980
T.L. Dormandy
Free radical reactions in biological systems
Annals of the Royal College of Surgeons of England, 62 (1980), pp. 188-194
View Record in ScopusGoogle Scholar

Duncan, 1957
B.D. Duncan
Multiple range tests for correlated and heteroscedastic means
Biometrics, 13 (1957), pp. 359-364
Google Scholar

Elgawish et al., 1996
A. Elgawish, M. Glomb, M. Friedlander, V.M. Monnier
Involvement of **oxide** in collagen cross-linking by high glucose in vitro and **in vivo**
Journal of Biological Chemistry, 271 (1996), pp. 12964-12971
ArticleDownload PDFView Record in ScopusGoogle Scholar

Ellman, 1959
G.L. Ellman
Tissue sulphydryl groups
Archives of Biochemistry and Biophysics, 82 (1959), pp. 70-77
ArticleDownload PDFView Record in ScopusGoogle Scholar

Fang et al., 2002
Y.Z. Fang, S. Yang, G. Wu
Free radical, antioxidant, and nutrition
Nutrition, 18 (2002), pp. 872-890
View Record in ScopusGoogle Scholar

Halliwell and Gutteridge, 1994
B. Halliwell, J.M.C. Gutteridge
Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy
Lancet, 1 (1994), pp. 1396-1397
View Record in ScopusGoogle Scholar

Haugaard, 1968
N. Haugaard
Cellular mechanisms of oxygen toxicity
Physiological Reviews, 48 (1968), pp. 311-373
CrossRefView Record in ScopusGoogle Scholar

Hong et al., 2004
J. Hong, M. Bose, J. Ju, J. Ryu hm, X. Chenm, S. Sang
Modulation of arachidonic acid metabolism by curcumin and related ß-diketone derivatives: Effects on cytosolic phospholipase A2, cyclooxygenases and 5-lipoxygenase
Carcinogenesis, 25 (2004), pp. 1671-1679

View Record in ScopusGoogle Scholar

Huebschmann et al., 2006
A.G. Huebschmann, J.G. Regensteiner, H. Vlassara, J.E. Reusch
Diabetes and advanced glycoxidation end products
Diabetes Care, 29 (2006), pp. 1420-1432
CrossRefView Record in Scopus Google Scholar

Hunt, 1996
J.V. Hunt
Ascorbic acid and **DM**
Subcellular Biochemistry, 25 (1996), pp. 369-405
CrossRecord in Scopus Google Scholar

Ihara et al., 1999
Y. Ihara, S. Toyokuni, K. Uchida, H. Odaka, T. Tanaka, H. Ikeda
Hyperglycemia causes oxidative stress in pancreatic beta cells of GK rats a model of type 2 diabetes
Diabetes, 48 (1999), pp. 927-932
View Record in ScopusGoogle Scholar

Ingold et al., 1987
K.U. Ingold, A.C. Webb, D. Witter, G.W. Burton, T.A. Metcalfe, D.P. Muller
Vitamin E remains **the key** lipid-soluble, chain-breaking antioxidant in human plasma even in individuals **stricken by** severe **vitamin E** deficiency
Archives of Biochemistry and Biophysics, 259 (1987), pp. 224-225
Article Record in Scopus Google Scholar

Jiang et al., 1992
Z.Y. Jiang, J.V. Hunt, S.D. Wolff

Ferrous ion oxidation **within the** presence of xylenol orange for detection of lipid hydroperoxide in **lipoprotein**
Analytical Biochemistry, 202 (1992), pp. 384-389
View Record in Scopus Google Scholar

Jin et al., 2008
L. Jin, H.Y. Xue, L.J. Jin, S.Y. Li, Y. Xu
Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes
European Journal of Pharmacology, 582 (2008), pp. 162-167
ArticleDownload PDFView Record in ScopusGoogle Scholar

Joe et al., 2004
B. Joe, M. Vijaykumar, B.R. Lokesh
Biological properties of curcumin cellular and molecular mechanisms of action
Critical Reviews in Food Science and Nutrition, 44 (2004), pp. 97-111
View Record in ScopusGoogle Scholar

Johansen et al., 2005
J.S. Johansen, A.K. Harris, D.J. Rychly, A. Ergul
Oxidative stress **and also the** use of antioxidants in diabetes: Linking basic science to clinical practice
Cardiovascular Diabetology, 4 (2005), p. 5
CrossRefView Record in ScopusGoogle Scholar

Karpen et al., 1982
C.W. Karpen, K.A. Pritchard, A.J. Merola, R.V. Ranganamala
Alternations of the prostaglandin–thromboxane ration in streptozotocin-induced diabetic rats
Prostaglandin Leukotnen Medicine, 8 (1982), pp. 93-103

ArticleDownload PDFView Record in ScopusGoogle Scholar

Karthikeyan et al., 2010
K. Karthikeyan, L. Pari, V.P. Menon
Combined treatment of tetrahydrocurcumin and chlorogenic acid exerts **a possible** antihyperglycemic effect on streptozotocin–nicotinamide-induced diabetic rats
General Physiology and Biophysics, 29 (2010), pp. 23-30
CrossRefView Record in ScopusGoogle Scholar

Kasai et al., 2000
H. Kasai, S. Fukada, Z. Yamaizumi, S. Sugie, H. Mori
The action of chlorogenic acid in vegetables and fruits as an inhibitor of 8-hydroxydeoxy guanosine formation in vitro and a rat carcinogenesis model
Food and Chemical Toxicology, 38 (2000), pp. 467-471
ArticleDownload PDFView Record in ScopusGoogle Scholar

Rhode et al., 2000
S.M. Khopde, K.I. Priyadarisini, S.N. Guha, J.G. Satav, P. Venkatesan, M.N. Rao
Inhibition of radiation-induced lipid peroxidation by tetrahydrocurcumin: Possible mechanism by pulse radiolysis
Bioscience, Biotechnology, and Biochemistry, 64 (2000), pp. 503-509
CrossRefView Record in ScopusGoogle Scholar

Kim et al., 2003
M.J. Kim, G.R. Ryu, J.S. Chung, S.S. Sim, D.J. Min Dos Rhie, S.H. Yoon, et al.
Protective effect of epicatechin against the toxic effects of STZ on rat pancreatic islets: **in vivo** and in vitro
Pancreas, 26 (2003), pp. 292-299
View Record in Scopus Google Scholar

AUTHOR AFFILIATION

FASEFAL PLIMAYESHU; KALEA AKILY