CAMBA, a New Synthesized and Promising Protector against STZ-Induced Diabetic Complications in Rats

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Abstract

CAPE, one of the most active compounds in propolis, is the perfect illustration of a natural compound exhibiting diverse biological activities. However, rapid decomposition by esterase leads to its low bioavailability in vivo. The aim of the present study is to synthesis and investigates the antioxidant and protective activities of novel derivative of caffeic acid, CAMBA against STZ-induced diabetic complications in diabetic rats. Since amide is more resistant to esterase enzyme. CAMBA was synthesized by reacting the amino group of methyl anthranilate with caffeic acid in the presence of PCl3. Diabetic rats was induced by injection of STZ (55 mg/kg, i.p.) and diabetes was confirmed 48 h after induction, and then allowed for 7 days to stabilize blood glucose level. CAMBA (25 and 50 mg/kg b.w daily for 28 days) treated diabetic rats significantly reduced elevated blood glucose, TC, TG, atherogenic index, LDL-c, vLDL-c, hepatic, renal and cardiac TBARs and HP. The treatment also resulted in improved the insulin and insulin resistance and significantly increased serum HDL-c and GSH, SOD, CAT and GPx in the liver, kidney and heart of diabetic rats. The results clearly suggest that CAMBA treated group may effectively normalize the impaired antioxidant status in streptozotocin induced diabetes than the glibenclamide-treated groups. CAMBA exerted rapid protective effects against lipid peroxidation by scavenging of free radicals by reducing the risk of diabetic complications. Taken together, CAMBA has potential as an antioxidant agent for diabetes and deserves clinical trial in the near future as an adjuvant therapy in diabetic patients.

Keywords: CAMBA; Antioxidant; STZ; Oxidative stress biomarkers and diabetic complications

Abbreviations: CAPE: Caffeic Acid Phenylethyl Ester; CAMBA: Caffeic Acid Methyl Benzoate Amide; TC: Total-Cholesterol; LDL-c: Low Density Lipoprotein-Cholesterol; vLDL-c: Very Low Density Lipoprotein-Cholesterol; HDL-c: High Density Lipoprotein-Cholesterol; TBARs: Thiobarbituric Acid Reactive Substances; HP: Hydroperoxides, GSH: Reduced Glutathione, SOD: Superoxide Dismutase, CAT: Catalase, GPx: Glutathione Peroxidase, GST: Glutathione-S-Transferase; STZ: Streptozotocin

Introduction

Diabetes mellitus (DM) represents a range of metabolic disorders characterized by hyperglycemia resulting from insulin deficiency or insulin resistance or both [1,2]. Complications caused by hyperglycemia involve damage to the small vessels such as in neuropathy, nephropathy, and retinopathy, and large blood vessels as in cardiovascular diseases [3-5]. STZ is potential to act as an intracellular nitric oxide (NO) donor and generates reactive oxygen species (ROS) [6,7]. The synergistic action of both NO and ROS may also contribute to DNA fragmentation and other deleterious changes caused by STZ [8]. Many natural polyphenolic compounds are demonstrated to have anti-inflammatory, antioxidant, anticarcinogenic, antithrombotic, and cardiovascular protective effects [9-11]. Caffeic acid, the main representative of the hydroxybenzonic and phenolic acids, is found in many plants as simple derivatives such as glycosides, amides, esters and sugar esters. Caffeic acid phenylethyl ester (CAPE) (Figure 1), one of the most active compounds in propolis, is the perfect illustration of a natural compound exhibiting diverse biological activities. Numerous studies have shown that CAPE has antioxidant, anti-inflammatory and hepatoprotective activities [12-14]. However, the rapid metabolism of CAPE by esterase leads to its low bioavailability.

The antioxidant activities of CAMBA are based on structural factors as well as the medium used to assay these activities [15]. Hussein [15] showed that free radical scavenging activity was dependent on the presence of catechol, hydroxyl groups and the H donating groups. Also, the hydrophobicity of CAMBA was also an important factor in determining their activity [15]. In addition to these known structural features, it was further demonstrated that phenolic acids bearing a carbonyl group separated from the aromatic ring were more active [15].

As an extension of our studies on the synthesis of some new biologically active heterocyclic compounds [15,16], now we wish to report the synthesis caffeic acid methyl benzoate amide (CAMBA), a caffeic acid amide derivative and structurally similar to CAPE. Since amide is more resistant to esterase, it is foreseeable that CAMBA is more stable than CAPE in vivo. The present investigation was undertaken to assess the effect of CAMBA on tissue lipid peroxides and enzymatic antioxidant in STZ-induced diabetic rats, which may pave the way for possible therapeutic application.

Experimental

Chemistry

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu MR470 infrared spectrophotometer using the KBr pellets. 1H- and 13C NMR spectra were recorded in DMSO-d6 and CD3OD, respectively, on a Varian EM 360 (1H NMR at 240 MHz) and 4832580; Fax: +2 (0122) 38353270; E-mail: abdallahmohammed_304@yahoo.com

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Mass spectra were run using HP Model MS-5988. Microanalytical data (C, H, N) were determined at the Microanalytical Center, Cairo University, Egypt. Synthesis of caffeic acid methyl benzoate amide (CAMBA): Caffeic acid methyl benzoate amide (CAMBA) was prepared as the method adapted by Hussein [15]. To a solution of caffeic acid (0.01 mol) and methylantranilate (2.31 g, 0.01 mol) in xylene (50 ml), phosphorus trichloride (3 ml) was added. The reaction mixture was heated under reflux for 3-4 h. The crude product was recrystallized from ethanol to give CAMBA.

Animals

Male albino rats weighing around 230 ± 10 g were purchased from Faculty of Veterinary Medicine, Cairo University. The reason that potentiates us to use rats for testing is because they are simply easy to find, their blood and organs; liver, kidney and heart are enough to make all biochemical analysis. All the rats were given a period of acclimatization for 15 days before starting the experiment. Animals were provided with standard diet and water ad libitum and were kept on a 12 h light/12 h dark cycle, in a room with the temperature regulated to 21-25°C and humidity at roughly 56%. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as per the directions of Faculty of Pharmacy October 6 University, Egypt.

Acute toxicity studies

Acute oral toxicity of CAMBA study was performed as the method adapted by Hussein [15]. Administration of stepwise dose of CAMBA (400, 800 and 1000 mg/kg b.wt), animals were observed individually during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for total of 21 days. The parameters observed were growing, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [17].

Induction of diabetes

STZ-induced diabetes has been described as a useful experimental model to study the activity of hypoglycemic agents [18,19]. After an overnight fasting (deprived of food for 16 hours had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ (Sigma, St. Louis, Mo. dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 55 mg/kg body weight) [20]. After 6 h of STZ injection, rats were received 5 % dextrose solution for the next 24 h to prevent STZ induced fatal hypoglycemia as a result of massive pancreatic insulin release after its administration [21]. Diabetes was confirmed 48 h after induction by measurement of tail vein blood glucose levels using glucose oxidase-peroxidase method. Diabetic rats were kept 7 days under standard laboratory condition for the stabilization of blood glucose levels [22]. After a week time for the development of diabetes, the rats with moderate diabetes having glucosuria and hyperglycemia (blood glucose range of above 15.6 mM/L) were considered as diabetic rats and were used for the further experiments. The change in the body weight experimental animals was observed throughout the treatment period.

Experimental set up

The rats were divided into seven groups with eight rats in each.

Group I: normal control rats received propylene glycol, orally (5 mL/kg).

Group II: Normal control rats were treated with CAMBA (25 mg/kg) [15].

Group III: Normal control rats were treated with CAMBA (50 mg/kg) [15].

Group IV: Diabetic control (STZ-induced diabetic rats, was given propylene glycol, orally (5 mL/kg).

Group V: STZ-induced diabetic rats were treated with CAMBA (25 mg/kg) [15].

Group VI: STZ-induced diabetic rats were treated with CAMBA (50 mg/kg) [15].

Group VII: STZ-induced diabetic rats were treated with glibenclamide (5 mg/kg) [23].

CAMBA and glibenclamide were suspended in propylene glycol and administered orally to its respective group animals for 28 days. The fasting blood glucose level and body weight were estimated every week (0, 7, 14, 21 and 28 day). At the end of the fourth week, the fasted rats were sacrificed by cervical decapitation and the blood was collected using sodium fluoride as anticoagulant for determination of blood glucose. The liver and kidneys were dissected out, washed in ice-cold saline, patted dry and weighed.

Blood sampling and biochemical assays

The blood samples, centrifuged, and plasma was used freshly for estimation of plasma glucose [24]. Homeostatic index of insulin resistance (HOMA-IR), calculated by glucose (mM) x insulin (µU/ml)/22.5 [25]. Triacylglycerols [26], total cholesterol [27], HDL-cholesterol [28], LDL-cholesterol [29] formula (LDL-cholesterol=total cholesterol−triacylglycerols−5×HDL-cholesterol). VLDL-cholesterol concentration [30] formula (vLDL-cholesterol=triacylglycerols/5). The atherogenic index [log (TG/HDL-C)] was also calculated [31]. A portion from liver, Kidney and/or heart was blotted, weighed and homogenized with methanol (3 volumes). It was used for the estimation of TBARs [32] and hydroperoxides [33]. Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of GSH [34], GPx [35], Catalase (CAT) [36], SOD [37] and protein content [38].

Measurement of lipid peroxidation

A thiobarbituric acid reactive substances (TBARS) assay kit (ZepptoMetrix) was used to measure the lipid peroxidation products, malondialdehyde (MDA) equivalents [39]. In brief, liver, renal and heart tissues were homogenized with 0.1 mol/l sodium phosphate buffer (pH 7.4). One hundred microliters of homogenate were mixed with 2.5 ml reaction buffer (provided by the kit) and heated at 95°C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The lipid peroxidation products are expressed in terms of MDA equivalents.

Measurement of antioxidant enzymes

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase (CAT) activities were determined using commercially available

![Figure 1: Caffeic acid phenylethyl ester (CAPE).](http://dx.doi.org/10.4172/2161-0444.1000153)
assay kits (Biodiagnostic). Briefly, liver, renal and heart tissues were weighed and homogenized with appropriate buffers (provided by the kits). The homogenates were then determined following the procedures provided by the respective manufacturers. The superoxide dismutase assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by red formazan dye reduction produced [40]. One unit (U) of SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The glutathione peroxidase assay kit measures GPx activity indirectly by a coupled reaction with glutathione reductase (GR) [41]. Oxidized glutathione, produced upon reduction of hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A340 is directly proportional to the GPx activity. One unit (U) of GPx activity is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP+ per minute at 25°C. The specific activities of the various enzymes in the rat liver, renal and heart are expressed in U/mg of the protein with the protein content determined as stated above.

The catalase assay kit utilizes the peroxidative function of CAT for determination of enzyme activity [42]. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The generated formaldehyde is assayed spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as the chromogen. One unit (U) of CAT activity is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C.

Statistical analysis

All data were expressed as mean ± SD. All analyses utilized SPSS 15.0 statistical package for Windows (SPSS Inc., Chicago, IL). A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value 0.05 was accepted as statistically significant. Diabetic control rats were compared with normal control rats as well as CAMBA and glibenclamide treated diabetic rats.

Results

Chemistry

The synthesis of the target compound caffeic acid methyl benzoate amide (CAMBA) was achieved by the route depicted in Scheme 1.

Physicochemical and analytical data are listed in Table 1. IR (KBr, cm⁻¹) CAMBA: 3332 cm⁻¹ (OH), 3133 cm⁻¹ (NH), 2962 cm⁻¹ (CH-arom.), 2854 cm⁻¹ (CH-aliph.), 1715, 1685 (2C=O), 1520 cm⁻¹ (C=N). MS (m/z) CAMBA: 313 (M+, 10.57%), 276 (10.25%), 224 (14.29%), 183 (21.42%), 137 (25.05%), 97 (100%), 55 (46.42%). ¹H-NMR (DMSO-d6) CAMBA: 2.4 [s, 3H, COCH₃], 6.1-6.2 and 7.3-7.4 [d, 2H, CH=CH, two trans-olefinic protons], 6.7-8.0 [m, 7H, Ar-H], 10.2 [s, 1H, NH]. ¹³C-NMR(75MHz, CD₃OD) CAMBA:140.322(C-1), 121.392(C-1'), 123.303(C-2),115.529(C-2'), 130.500(C-3), 145.452(C-3'), 125.521(C-4), 145.702(C-4'), 129.859(C-5), 115.929(C-5'), 119.102(C-6), 165.021(C-7), 144.63(C-7'), 55.520(C-8), 114.663(C-8'), 199.47(C-9').

Antidiabetic and Antioxidant Activity of CAMBA

Acute toxicity study

In rats, oral administration of CAMBA at a dose of 1000 mg/kg did not produce any signs of toxicity and no animals were died up to 21 days. It showed that CAMBA was nontoxic in rats up to an oral dose of 1000 mg/kg b.w. Hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion were not observed at all used doses. Therefore, investigation of hypoglycemic and antioxidant activity was carried out using 25 and 50 mg/kg dose levels.

Effect of CAMBA on body weight, blood glucose, insulin and HOMA-IR levels

Administration of STZ significantly (P<0.01) reduced body weight in diabetic rats compared to normal control rats (Table 1). In diabetic rats, treatment of both doses of CAMBA and glibenclamide significantly (P<0.01) increased body weight compared to diabetic control rats. The blood glucose and HOMA-IR levels were significantly (P<0.01) increased but decreased the level of insulin (P<0.01) after the administration of STZ compared to the normal control rats. Oral treatment of CAMBA 25 & 50 mg and glibenclamide (5 mg/kg) decreased significantly (P<0.01) blood glucose and HOMA-IR level as well as the level of plasma insulin was significantly increased (P<0.01) in diabetic rats compared to diabetic control rats (Table 2).

Effect of CAMBA on lipid profiles

In STZ-induced diabetic rats, TC, TG, LDL, vLDL and atherogenic index levels were increased and HDL level was decreased significantly (P<0.01) compared to normal control rats. In diabetic rats, administration of CAMBA 25 and 50mg/kg dose showed significant (P<0.01) reduction in elevated TC, TG, LDL, vLDL and atherogenic index levels compared to diabetic control rats. Also, a significantly (P<0.01) increased level of HDL was observed in diabetic rats treated with both doses of CAMBA and glibenclamide compared to diabetic control rats (Table 3).

Antioxidant activity of CAMBA

Table 4 shows the concentration of TBARS and hydroperoxides in liver, kidney and heart of control and experimental groups of rats. The levels of TBARS and hydroperoxides in diabetic rats were significantly higher (P<0.01) than control rats, whereas diabetic rats treated with CAMBA 25 and 50 mg/kg and glibenclamide restored the altered values to the near normalcy. The antioxidant activity of CAMBA in liver, kidney and heart was studied in diabetic rats and the data were given in Table 5. After the induction of diabetes by STZ, significantly (P<0.01) decreased levels of GSH, GPx, SOD and CAT were observed compared to normal control rats. These altered above antioxidant levels were reversed significantly (P<0.01) to near normal levels after the administration of CAMBA 25 and 50 mg/kg dose and glibenclamide 5 mg/kg dose compared to diabetic control rats.

Discussion

Our study addresses the protective effect of CAMBA against STZ-induced diabetic complications in rats. CAMBA was synthesized according the method adapted by Hussein, 2013 and its structure was proved on the basis of elemental analyses, IR, mass and HNMR spectral data. The IR spectrum of compound CAMBA showed bands at 3332 cm⁻¹ (OH), 3133 cm⁻¹ (NH), 2962 cm⁻¹ (CH-arom.), 2854 cm⁻¹ (CH-aliph.), 1715, 1685 (2C=O), 1520 cm⁻¹ (C=N). Mass spectrum of CAMBA showed peaks corresponding to C₂₃H₃₄O₉N₂ at m/z 441.2772 (found 441.2788). The elemental analysis of CAMBA was found to be: C 66.20, H 6.20, N 4.47.

| Compd. No. | M.P. [C] | Yield (%) | Mol. Formula (Mol. Wt.) | Elemental analyses Calcd./Found [%] |
|------------|---------|-----------|------------------------|----------------------------------|
| CAMBA      | 178-180 | 77        | C₃₉H₃₄O₉N₂ (313)        | C 66.20 H 6.20 N 4.47 |

Table 1: Physico-chemical properties and molecular formulae of CAMBA.
compound CAMBA exhibited molecular ion peak 313 (M+ , 10.57%) with base peak at 97 (100%) and other significant peaks at 276 (10.25%), 224 (14.29%), 183 (21.42%), 137 (25.05%) and 55 (46.42%). 1H NMR spectrum of compound CAMBA in (DMSO-d6) exhibited signals at 2.4 [s, 3H, COCH3], 6.2 [d, 2H, CH=CH, two trans-olefinic protons], 6.7–8.0 [m, 7H, Ar–H], 10.2 [s, 1H, NH]. Also, 13C-NMR spectrum of compound CAMBA in (CD3OD) exhibited signals at 144.63(C-7′), 109.21(C-8′) and 199.47(C-9′) indicated that the presence of double bond between C-7′ and C-8′ as well as precence of (C=O) group at (C-9′).

In acute toxicity study, CAMBA did not show significant toxicity signs when observed for the parameters during the first four hours and followed by daily observations for 21 days and no mortality was also...
The present study was conducted to evaluate the beneficial effects of CAMBA on antioxidant status in STZ-induced diabetic rats. The preliminary studies conducted by this work revealed the non-toxic nature of CAMBA on normal rats. Although no literature data have given any insight into the possible toxicological potentials of CAMBA, except for antioxidant and anti-inflammatory activity of CAMBA [15]. Hussein [15], reported the structure antioxidant relationship of CAMBA as well as its LD₅₀, which equal 1135 mg/kg.b.w. In fact, administration of CAMBA at 25 and 50 mg/kg.b.w. did not cause evident signs of liver, renal and heart injury as indicated by the activities of tissue oxidative stress biomarkers.

STZ [2-deoxy-2-[3-methyl-3-nitrosoureido]-D-glucopyranose] is commonly used to induce experimental diabetes in animals [6]. STZ-induced diabetes may be due to vitiate glucose oxidation and reduction of insulin biosynthesis and secretion. The toxicity of STZ is due to DNA alkylation of its methyl nitrosourea moiety mainly at 6 O-position of guanine [7]. The transfer of methyl group from STZ to the DNA molecule causes damage which results in fragmentation of DNA and functional defects of the beta cells. Moreover, STZ is potential to act as an intracellular nitric oxide (NO) donor and generates reactive oxygen species (ROS). The synergistic action of both NO and ROS may also contribute to DNA fragmentation and other deleterious changes caused by STZ [8].

Single dose of STZ (55 mg/kg.b.w. rats) in sterile citrate buffer (e.g. pH 4.5, 0.1 M) administered intraperitoneally [20]. The rats were allowed to resume feeding and drinking of 5% glucose solution for 30 minutes after the STZ administration to avoid the exposure of rats to a sudden drop in the level of insulin. Diabetes develops gradually and assessed after a seven days. In the present study, a plasma glucose level of about 13.8 mM indicates the induction of diabetes mellitus.

This model permits the evaluation of the effect of CAMBA in an animal without the interference of the side effects induced by STZ [43]. In our study, elevated blood glucose level and decreased insulin level were observed in STZ-induced diabetic rats and it may be due to above stated mechanism of STZ. Oral administration of CAMBA 25, 50mg/kg and glibenclamide to the diabetic rats significantly reduced blood glucose level with compared to diabetic control rats. Also, the decreased insulin and insulin resistance levels were noticed in diabetic rats compared to normal control rats which directly support and represent STZ-mediated β-cells destruction or damage. In diabetic rats, treatment of CAMBA and glibenclamide increased the insulin and insulin resistance levels compared to diabetic control rats. Hence, the hypoglycemic activity of CAMBA may be due to its protective action against STZ-mediated damage to the pancreatic β-cells and also possibly because of regeneration of damaged beta cell or increased insulin release or secretion.

Muscle wasting is an unintentional loss of body weight due to accelerated muscle proteolysis, resulting in loss of body cell mass. Insulin is an important regulator of protein synthesis and proteolysis in skeletal muscle. Insulin resistance or deficiency produces impaired muscle protein turnover and muscle wasting. The uncontrolled diabetes is associated with severe muscle wasting [44]. In this study, STZ-induced diabetes is characterized by severe loss in body weight [45] and this was also seen in the present study. CAMBA 25, 50 mg/kg and glibenclamide administration controlled this loss in body weight. However, it did not normalize the body weight completely as it remained lesser than normal control rats. The decrease in body weight observed in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source [22].

In recent years, considerable interest has been directed towards the investigation of plasma lipids and lipoproteins pattern in diabetes mellitus due to the fact that abnormal lipid level leads to the development of coronary artery disease in diabetic patients [46]. Reduced insulin secretion and defect in insulin function results in enhanced metabolism of lipids from adipose tissue to the plasma. Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated cardiovascular risk in diabetes mellitus [47]. Thus, the altered lipid and lipoprotein pattern observed in diabetic rats could be due to defect in insulin secretion and/or action. Accumulation of cholesterol and phospholipids in liver due to elevated plasma free fatty acids has been reported in diabetic rats [48]. Hyperglycemia is an important contributor for the cardiovascular diseases (CVD) risk.

The animal studies revealed that hyperglycemia produces glycation and peroxidation of proteins which cause damage to the arterial walls [49]. The prevalence of all forms of CVD is 2-8 folds higher in diabetic person compared to non-diabetic person. The accelerated coronary heart disease (CHD) has emerged as a leading cause of morbidity and mortality in diabetic patients in the worldwide [50]. The vascular diseases occurred in diabetes due to disturbance in lipoprotein metabolism which causes acceleration of atherosclerosis [48].

In the present study, increases in plasma TC, TG, LDL, vLDL and atherogenic index and reduced level of HDL levels were observed in STZ- induced diabetic rats. It is interesting to note that CAMBA did not only lower the TC, TG, LDL and vLDL levels, but also enhanced the cardio protective lipid HDL and atherogenic index of the diabetic rats after 30 days of treatment. The increased in HDL-cholesterol is a desirable feature. In addition, the reductions in TC, TG and LDL-cholesterol could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics [51]. This would
definitely reduce the incidence of coronary events being the major cause of morbidity and deaths in diabetes subjects [52]. HDL-C transports cholesterol from peripheral tissues to the liver, thereby reducing the amount stores in tissue and decreasing the likelihood of getting atherosclerotic plaques [53].

This study indicated that CAMBA 25, 50 mg/kg significantly reduced TC, TG, LDL, vLDL and atherogenic index and increased HDL concentrations (Table 3), which could be due to stimulating effect on insulin secretion from pancreatic β - cells (Table 2). The possible mechanism by which CAMBA can exert lipid lowering activities may be explained by decreasing the cholesterol biosynthesis, particularly by decreasing the activity of hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase or by reducing the NADPH required for cholesterol synthesis and/or by stimulating glucose utilization [54]. In our study, it has been proposed that CAMBA and glibenclamide acted in a similar way by increasing insulin production in STZ-induced diabetic rats and lowering TG level by activation of the enzyme lipoprotein lipase, because insulin activates lipoprotein lipase [55].

Several studies have demonstrated the involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes [56]. It has been reported that in diabetes mellitus, oxygen free radicals are generated by stimulating H2O2 in vitro as well as in vivo and in pancreatic β-cells [56]. The increased lipid peroxidation in the diabetic animals may be due to the observed remarkable increase in the concentration of TBARs and increased lipid peroxidation in the diabetic animals may be due to diabetes mellitus, oxygen free radicals are generated by stimulating lipid peroxidation during diabetes [56]. It has been reported that in diabetic rats and lowering TG level by activation of the enzyme acting in a similar way by increasing insulin production in STZ-induced pancreatic β-cells [56]. The result of Hussein [15] indicated that antioxidant activity of CAMBA due to the above two mechanisms electron transfer (ET) and hydrogen atom transfer (HAT). On the other hand, a consistent increase of antioxidant activity is reported with CAMBA where the carbonyl is indirectly linked to the aromatic ring [59]. Spacing between the carbonyl group and catechol aromatic ring (B) induces increase in potency as shown with CAMBA as well as reducing the space linked of amide group with the non-catechol aromatic ring (A) increases the antioxidant activity.

Antioxidant and protective effect of newly synthesized CAMBA against STZ-induced diabetic complications in rats has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind. Conclusively, our observations have clearly demonstrated that the CAMBA significant antioxidant and anti-hyperglycaemic activity due to its possible multiple effect involving both pancreatic and extra-pancreatic mechanism. CAMBA possessed a capability to inhibit the lipid peroxidation and activate the antioxidant markers (GSH, GPx, SOD and CAT) in diabetes. The ability to reduce oxidative stress may help to prevent diabetic complications. More studies are needed to prove its medicinal and biological importance which may pave the way for possible therapeutic applications.

| Groups | Treatment/Dose (mg/ml) | Liver | Kidney | Heart |
|--------|------------------------|-------|--------|-------|
|        | GSH (mg/100g tissue) | GPx U/mg protein | SOD U/mg protein | CAT U/mg protein | SOD U/mg protein | CAT U/mg protein | GSH (mg/100g tissue) | GPx U/mg protein | SOD U/mg protein | CAT U/mg protein |
| Normal Control | 142.34 ± 5.261 | 17.14 ± 3.71 | 13.89 ± 3.71 | 65.19 ± 4.71 | 101.00 ± 6.38 | 8.06 ± 1.43 | 10.44 ± 2.71 | 43.28 ± 3.48 | 115.48 ± 9.04 | 13.59 ± 2.50 | 9.08 ± 1.91 | 27.46 ± 2.83 |
| Control + CAMBA (25mg/kg) | 139.68 ± 7.90 | 16.05 ± 3.22 | 12.44 ± 1.75 | 60.34 ± 5.38 | 98.07 ± 5.40 | 8.11 ± 2.07 | 10.25 ± 2.27 | 46.08 ± 5.23 | 114.50 ± 6.37 | 13.33 ± 2.01 | 10.26 ± 1.79 | 29.04 ± 5.26 |
| Control + CAMBA (50mg/kg) | 140.57 ± 8.11 | 18.10 ± 4.20 | 13.06 ± 7.11 | 64.35 ± 10.71 | 104.71 ± 5.09 | 8.98 ± 1.90 | 11.17 ± 2.39 | 46.69 ± 7.08 | 117.92 ± 10.25 | 14.05 ± 3.27 | 12.74 ± 3.08 | 28.11 ± 3.29 |
| Diabetic Control | 87.45 ± 5.08 *** | 7.44 ± 1.15 *** | 4.19 ± 1.33 *** | 37.94 ± 4.38 *** | 55.06 ± 4.38 | 4.56 ± 0.58 *** | 5.94 ± 1.47 *** | 27.93 ± 4.66 *** | 76.82 ± 6.08 *** | 5.99 ± 1.85 *** | 6.55 ± 1.13 *** | 15.95 ± 2.18 *** |
| Diabetic + CAMBA (25mg/kg) | 122.73 ± 6.46 ** | 11.82 ± 2.00 ** | 7.86 ± 2.35 ** | 50.08 ± 6.26 | 75.97 ± 6.84 | 6.27 ± 1.08 ** | 7.16 ± 0.77 ** | 35.00 ± 4.86 ** | 84.26 ± 4.75 ** | 8.22 ± 2.04 ** | 7.72 ± 1.94 ** | 22.49 ± 3.30 ** |
| Diabetic + CAMBA (50mg/kg) | 138.83 ± 7.93 ** | 15.77 ± 2.57 ** | 11.46 ± 3.24 ** | 59.63 ± 4.81 | 89.44 ± 7.65 | 9.63 ± 2.48 ** | 10.39 ± 2.71 ** | 41.60 ± 4.22 ** | 104.13 ± 7.38 ** | 11.29 ± 2.41 ** | 10.55 ± 2.03 ** | 25.37 ± 4.02 ** |
| Diabetic + Glibenclamide (5mg/kg) | 128.44 ± 6.50 ** | 12.85 ± 2.18 ** | 9.59 ± 3.99 | 54.16 ± 5.07 | 80.39 ± 8.15 | 9.80 ± 2.37 | 30.85 ± 2.79 | 90.44 ± 8.06 | 9.08 ± 2.11 | 9.12 ± 1.60 | 19.70 ± 3.46 ** |

Values represent the mean ± SE (n=8). Experimental groups were compared with the diabetic control rats. Activity is expressed as: 50% of inhibition of epinephrine auto oxidation per min for SOD; μmoles of hydrogen peroxide decomposed per min for catalase; μmoles of glutathione oxidized per min. for GPx; **significantly different from control group at p<0.01

Table 6: Effect of CAMBA on liver, kidney and heart GSH, GPx, SOD and CAT in control and experimental groups of rats.
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