The (-)-epigallocatechin gallate (EGCG) ameliorates hyperglycemia and hyperlipidemia - mediated oxidative stress in liver and kidney of alloxan induced diabetic mice

Ahlem SOUSSI
university of sfax
Manel GARGOURI (manele.gargouri@gmail.com)
university of sfax
Sirine AOUEDI
university of sfax
Amel AKROUTI
university of sfax
Christian MAGNE
university of Western Brittany
Abdelfattah EL FEKI
university of sfax

Research Article

Keywords: (-)-epigallocatechin gallate, Alloxan, Diabetes, Oxidative stress, Antioxidant enzymes, Lipid peroxidation

Posted Date: May 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-28586/v1

Abstract

Background: Diabetes mellitus has become a serious problem associated with health complications, such as metabolism disorders and liver-kidney dysfunctions. The inadequacies associated with conventional medicines have led to a determined search for alternative natural therapeutic agents. The present study is conducted to evaluate the hypoglycemic, antilipidemic and antioxidant effects of EGCG in surviving diabetic mice.

Methods: Alloxan diabetic mice were injected with EGCG. Their bloods were collected and submitted to various biochemical measurements, including blood glucose, cholesterol, triglycerides, urea, creatinine and transaminases. Their livers and kidneys were isolated to perform histological analysis.

Results: Both EGCG and insulin treatments of diabetic mice resulted in a significant reduction in fasting blood glucose level. EGCG supplementation also ameliorated hepatic as well as renal toxicity indices. Moreover, diabetic mice injected by EGCG exhibited significant changes in antioxidant enzyme activities in the liver and kidney. Histological analyses also showed that it exerted an ameliorative action on these organs and efficiently protected the liver-kidney functions of diabetic mice.

Conclusion: The findings revealed that EGCG administration induced attractive curative effects on diabetic mice, particularly in terms of liver-kidney functions. EGCG can, therefore, be considered as a potential strong candidate for future application to treat and prevent diabetes.

Introduction

Diabetes is one of the leading chronic diseases related to oxidative stress. This disease is characterized by chronic hyperglycaemia resulting from a defect in the secretion or action of insulin. It is associated in the long term with macroangiopathic and microangiopathic complications. The classic symptoms of diabetes are defined as: hunger and thirst with increased urine volume, thinness or obesity and risk of coma. Some studies have shown that in the case of type 1 diabetes, oxidative stress causes insular destruction of the pancreas, either by necrosis or apoptosis of the beta cell [1]. Indeed, four main molecular mechanisms have been implicated in this oxidative stress-induced tissue damage: activation of protein kinase C (PKC) isoforms, increased flow of the hexosaminase pathway, glycation. There has been an explosive trend in antidiabetic herbal medicine that is currently experiencing considerable growth because of the discovery of a large number of antidiabetic and antioxidant plant extracts.

In fact, numerous studies have made it possible to isolate bioactive molecules from plants that could protect the body against diabetes mellitus and the associated oxidative stress, namely polyphenols, which are widespread in nature and present in plants. Fruits, vegetables, wine [2] and especially medicinal plants namely walnut, green tea and mallow [3], which are considered among the most important exogenous natural antioxidants. In addition, flavonoids are known for their antioxidant, antibacterial, antidiabetic, antiviral and anti-inflammatory properties [4]. Appreciated for ages, green tea, a widespread drink in Tunisia, from the plant *Camellia sinensis,* contains a host of active ingredients, including polyphenols, namely catechins, which have attracted the attention of scientists. The scientific community's interest in the beneficial effects of tea is more directed towards the most abundant catechin in green tea, epigallocatechin–3-gallate (EGCG).

The present study was designed to evaluate "in vitro" the antioxidant power of the EGCG molecule through the application of total antioxidant activity, DPPH, FRAP, NO• and the antihyperglycemic and antihyperlipidemic powers of EGCG by studying the activities of α-amylase and lipase. To study "in vivo" the impact of an exposure to alloxan associated or not with EGCG by the assay of the various parameters of the oxidative status and this during 15 days. To study the histopathological profile of the liver and kidney in order to confirm the protective effect of EGCG against the glycaemic status as well as the oxidative and enzymatic processes following the injection of alloxan.

Material And Methods

Reagents

All reagents used in this study were purchased from Sigma Chemicals Co. (St. Louis, MO, USA): Alloxan, Folin-Ciocalteu phenol reagent, 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB), L-Glutathione (reduced form) and thiobarbituric acid (TBA). Insulin kit was provided by Gentaur, France.

Laboratory animals and experimental diabetes induction

Swiss mice (supplied by Tunisia Central Pharmacy), weighting about 30 g, were divided into batches of 6 animals. Animals were kept in a breeding farm at 22 °C with a stable hygrometry, under constant (10 h darkness/24 h) photoperiod. They have been fed on 14%-proteins commercial food pellets (Sico, Sfax, Tunisia. Animals were maintained in accordance with the guidelines for animal care approved by the Science Faculty of Sfax (Tunisia).

Mice were injected intraperitoneally with a freshly prepared solution of alloxan monohydrate in sterile normal saline (0.9% sodium chloride) at a dose of 120 mg/kg of body weight [5]. Because alloxan is able to induce fatal hypoglycaemia as a result of massive pancreatic insulin release, mice were treated with 20% glucose solution (5 to 10 mL) orally 6 hours post alloxan administration. The mice were then kept for the next 24 h with 5% glucose solution bottles in their cages to prevent hypoglycaemia [6]. After 2 weeks, mice with moderate diabetes having glycosuria and hyperglycaemia (i.e., with blood glucose levels of 200 to 300 mg/dL) were chosen for the experiment.

Experimental design

Mice were divided into 4 groups: Control group (C), Diabetic mice group (D), Diabetic mice treated with insulin injections and given distilled water as sole beverage group (D+I) and Diabetic mice treated by EGCG group (D+E). (C) and (D) groups received distilled water at the same period. At day 15 eight

animals from each group were rapidly sacrificed by decapitation in order to minimize the handling stress. Liver and kidney were rapidly removed, cleared from fat, and frozen at –20 °C until use. Blood plasma was recovered and used for the estimation of glucose levels.

Evaluation of in vitro antioxidant effects

Determination of DPPH radical scavenging activity

The free radical scavenging activity of the EGCG was determined by the 1,1 Diphenyl–2-picrylhydrazyl (DPPH) assay described by Koleva et al. (2002) [7]. Inhibition of free radical DPPH, in percentage was calculated as: Scavenging activity (%) = ([A0 - A1]/A0] × 100.

A_0 is absorbance of blank at 517 nm and A_1 is the absorbance of the sample in the presence of the extract. IC_{50} in this test was defined as the concentration of EGCG that was able to inhibit 50% of the total DPPH radicals.

Determination of total antioxidant capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method following [8]. Extract (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min and cooled to room temperature. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (UVmini–1240) against blank after cooling to room temperature. Methanol (0.3 mL) as a control. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by dissolving ascorbic (1000; 500; 250; 125; 62.5 and 31.25 µg/mL) in methanol.

Determination of Fe^+ chelating activity

The reducing power was determined according to the method of Megías et al. (2009) [9]. Sample (1 mg/mL) was mixed with 1 mL of 200 mM sodium phosphate buffer (pH = 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 1 mL of 10% trichloroacetic acid (w/v) was added. The mixture was centrifuged at 2000 rpm for 10 min. The upper layer solution (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of fresh ferric chloride (0.1%). Absorbance was measured at 700 nm; a higher absorbance was taken to indicate a higher reducing power.

Determination of NO˙ Chelation activity

Nitric oxide was produced by the spontaneous decomposition of sodium nitroprusside (20 mM) in phosphate buffer (pH = 7.4). Once NO is generated, it interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. The entrapment of nitric oxide (NO •) can be determined by the method described by Shirwaikar et al. (2006) [10]. A solution of sodium nitroprusside (20 mM) was prepared in phosphate buffer (0.5 M, pH = 7.4). The reaction mixture is composed of 2 ml (20 mM) and 250 µl of each extract, and incubated at 25° C for 150 min. After incubation, a volume of 1 ml of each solution was taken and diluted with 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO and 0.1% N–1-anphthyl ethylenediamine). The mixture is again incubated for 30 min at room temperature (25° C), then the absorbance is measured at 546 nm against the blank. Ascorbic acid was used as standard. The percentage of NO trapping of the extract was calculated according to the following formula:

$NO^{\bullet}$ (%) = $[(A_0 - A_1)/ A_0] \times 100$

- A0: absorption of control
- A1: l'absorbance of « EGCG »

Estimation of seric glucose

Seric levels of glucose were determined using commercial diagnostic kit (Biomaghreb, Tunis, Tunisia). The absorbance was read at 620 nm. Glucose was expressed as milligrams per ml of blood.

Anti-diabetic activity of the EGCG

Anti-diabetic activity assay kit (REF 80023, Biolabo-France (Maizy, France)) was used to determine the amylase activity.

Anti-lipidimic activity of the EGCG

Anti-lipidemic activity assay kit (REF 95801, Biolabo-France (Maizy, France)) was used to determine the lipase activity.

Serum biochemical factors

The serum levels of lipids [total triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C)], liver enzymes [aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT), as well as urea and creatinine were measured by commercial kits purchased from Biomérieux Laboratory (Marcy-L'étoile, France) and Beckman Coulter Laboratory (Brea, CA).

Estimation of lipid peroxidation

The level of lipid peroxidation in animal tissues was estimated by measuring thiobarbituric acid reactive species (TBARS) according to Yagi (1976) [11]. About 1g of rat organs, namely liver and kidney, was cut into small pieces and immersed into 2 ml ice-cold lysis Tris-buffered saline (TBS, pH = 7.4). The mixture was then sonicated (10 seconds, twice) and centrifuged (5000*xg,* 30 min, 4°C). Supernatants were collected and stored at –80°C until use. For the assay, 125 µl of supernatants were homogenized by sonication with 50 µl of TBS, 125 µl of TCA-BHT in order to precipitate proteins and centrifuged (1000*xg;* 10 min; 4°C). 200 µl of the resulting supernatant were mixed, with 40µl of HCl (0.6M) and 160 µl of TBA (dissolved in Tris), and the mixture heated at 80°C for 10 minutes. The absorbance of the supernatant was then read at 530 nm. The amount of 2-thiobarbituric acid-reactive material (TBA-rm) was calculated using an extinction coefficient of 156 mM^{-1} cm^{-1}.

Antioxidant enzyme activities

Catalase activity was measured following the procedure of Aebi, 1984 [12]. The reaction mixture (1 mL) contained 100 mM phosphate buffer (pH = 7), 100 m M $H_2O_2$and 20µl (about 1–1.5 mg of protein) of liver homogenate. H_2O_2 decomposition was followed by measuring the decrease in absorbance at 240 nm for 1 minute. The enzyme activity was calculated using an extinction coefficient of 0.043 mM^{-1} cm^{-1} and expressed in international units (IU), i.e., in µmoles H_2O_2 destroyed /mn/mg of protein, at 25°C.

SOD activity was determined by measuring inhibition of photo reduction of NBT [13]. Briefly, one milliliter of supernatant was mixed with 50mM phosphate buffer (pH 7.8), 39mM methionine, 2.6 mM NBT and 2.7 mM EDTA. Riboflavin, at a final concentration of 0.26mM, was added at last and light switched on allowing the initiation of the reaction. Changes in absorbance at 560 nm were, then, recorded for 20 min. The activity was expressed as units/mg protein, at 25°C. In this assay, one unit of SOD is defined as the enzyme amount required to inhibit the photo reduction of NBT by 50%.

Glutathione peroxidase activity was measured according to Paglia (1967) [14]. One millilitre of 50 mM phosphate buffered saline (PBS) (pH 7.4) containing 5 mM Ethylene-diaminetetra-acetic acid (EDTA), 2 mM reduced -nicotinamide adenine dinucleotide phosphate (NADPH), 20 mM glutathione (GSH), 10 mM sodium azide (NaN_3), and 23 mU of oxidized glutathione (GSSG) reductase was incubated at 37°C for 5 min. Twenty microliters of 0.25 mM H_2O_2 solution and 10µl of sample (liver or kidney homogenate diluted 100 times) were added to the assay mixture. A change in absorbance at 340 nm was monitored for 1 minute. A blank control with all the ingredients except the sample was also monitored. The specific activity was calculated as millimoles of NADPH consumed per minute per milligram of protein (i.e. U/mg protein). Proteins were estimated by the method of Lowry et al. (1957) [15] using the bovine serum albumin (BSA) as the standard protein.

GSH Content

In the organ's homogenate, GSH content was estimated using a colorimetric technique, as mentioned by Ellman (1959) [16]. The assay is based on the development of a yellow color when DTNB [(5,5dithiobis-(2-nitrobenzoic acid)] is added. The absorbance at 412 nm was recorded and total GSH content was expressed as µmol GSH/g of liver or kidney.

Histological analyses

Classical procedures were used for histology. After fixation in Bouin solution, pieces of fixed tissue were embedded into paraffin, cut into 5µm slices and colored with hematoxyline-eosine.

Statistical analysis

For each group, data were presented as means ±SD of 6 animals. To compare between groups, we utilized one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test. Student unpaired t-test, used for comparison between 2 groups, was also utilized. Differences were considered significant at different levels (P <.05, P <.01, P <.001).

Results

Results of in vitro antioxidant assays

In this study, the antioxidant activity of EGCG was evaluated in vitro by using four antioxidant tests. The results of in vitro assays showed that EGCG had strong DPPH radical scavenging activity (IC_{50} = 4,64 µg/mL), moderate total antioxidant capacity (IC_{50} = 811.74 mg AAE g^{-1}DW) and relatively good ferrous iron chelating activity (IC_{50} = 0,645 µg/mL), and strong $NO^{\bullet}$ Chelation activity (IC_{50} = 0.12 mg/mL) (Table 1).

Results of laboratory animals

Blood glucose levels in diabetic and treated mice

The blood glucose level in the diabetic group was significantly higher than that in the control group (p <0.001). In diabetic mice treated with EGCG or insulin, about 68% and 82% decrease in glucose levels was observed respectively (Table 2).

Amylase inhibition assay

Our results show that EGCG has a glucose-lowering effect demonstrated by the decreased amylase activity by 47,7% in the presence of the molecule compared to the control.

Effects of insulin or EGCG on liver and kidney weights

According to the results, alloxan-treated mice exhibited significant liver weight loss over the 2 week period ($p < 0.05$). EGCG and insulin treatment also cause a variation in the liver weight but the difference was not statistically significant (Table 3).

Effects of insulin or EGCG on liver function

In alloxan- treated animals, the serum levels of AST, ALT, LDH and Bilirubin ($p < 0.001$) were significantly higher than control animals by respectively (137%, 72%, 17% and 70%) and treatment with insulin or EGCG significantly decreased elevation of these enzymes induced by alloxane (Table 4). Administration of EGCG in normal mice did not significantly change AST, ALT, LDH and Bilirubin levels compared to the control group (Data not schown).

Effects of insulin or EGCG on renal toxicity markers

Serum parameters of kidney function (urea and creatinine) in healthy, diabetic mice were significantly higher than control group (Table 4). Administration of EGCG significantly inhibited this elevation of renal markers levels induced by alloxan. EGCG showed no statistically significant differences compared to the control group (Table 4).

Effects of EGCG on lipid profile of alloxan-induced diabetic mice

As shown in table 5, the plasma TG, TC and HDL-C levels increased significantly in diabetic mice as compared to the control group. Administration of EGCG to diabetic mice significantly decreased these levels when compared to the diabetic group ($p < 0.05$,Table 3).

Lipase inhibition assay

Our results show that EGCG has an anti-obesity effect demonstrated by the decreased lipase activity by 30,6% in the presence of the molecule compared to the control.

Effects of insulin or EGCG on oxidative stress parameters

The liver and kidney tissues of alloxane-treated animals showed the significant elevation in the TBARS and $NO^{\cdot}$ levels in comparison with the control group ($p < 0.001$). However, administration of EGCG could significantly inhibit elevation of TBARS (liver and kidney; $p < 0.01$) level induced by alloxane. Furthermore, treatment with EGCG significantly reduced the $NO^{\cdot}$ level in liver and kidney tissues of alloxane-treated mice. Moreover, administration of EGCG by itself did not significantly change the level of TBARS and $NO^{\cdot}$ when compared to the control group (data not showen) (Figure 1).

Effects of insulin or EGCG on GSH level and antioxidant enzymes activities

The significant reduction was observed in the content of GSH and the activity of GPx, SOD and CAT in kidney and liver tissues of diabetic animals when compared to the control group ($p < 0.001$). On treatment with EGCG marked increase was observed in the level of GSH compared to the alloxan-treated group. Results indicated that the GSH content and antioxidant enzymes activities did not significantly change in tissues of normal animals administrated with EGCG (Figure 2 and 3).

Effects of insulin or EGCG on histological changes in liver and kidney tissues

Histological examinations of liver tissues showed that compared to the control group *(A),* the liver tissue ofalloxane-treated mice *(B)* showed severe damages which were observable in the form of focal degeneration of hepatocytes, dilation of liver sinusoids and high inflammatory cells diffusion around portal triad areas. *(C)* Insulin treated diabetic mice also had mild degeneration of hepatic tissues with existence of binucleated hepatocytes which as indication of remedy progress against diabetes induce liver pathology. *(D)* EGCG treated diabetic mice had normal hepatic architecture, although blood vessels congestion was noticed. Overall, protective effects of EGCG treatment on the mice' liver histopathology were general better than that of insulin mice (Figure 4).

Renal tissue treated with alloxane revealed severe renal injury and all types of kidney cells such as mesangial cells, podocytes and tubulo-interstive cells were affected and glomerular and tubular hypertrophy was also noticeable in addition to tubular dilatation *(B').* Insulin treatment of diabetic mice *(C')* reduced diabetes-induced dilation of the proximal tubules. The group of mice treated with EGCG *(D')* exhibited normal tubular and glomerular integrity with insignificant cellular infiltration (Figure 5).

Discussion

Currently, Diabetes mellitus is a major cause of disability and hospitalization. Available therapeutic options for non-insulin-dependent diabetes mellitus such as dietary modification, oral hypoglycemics and insulin have limitations of their own [17]. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future. A few reports are available on the hypoglycemic effects of EGCG.

Epigallocatechin–3-gallate (EGCG) is the main catechin flavonoid found in green tea (*Camellia sinensis)*.. It is a powerful antioxidant capable of neutralizing reactive oxygen species and free radicals involved in aging and chronic degenerative diseases. Indeed, EGCG is characterized by antitumor properties[18], antidiabetic agents [5], anti-inflammatory, and especially by antioxidant properties [19]. During this work, we tested *in vivo* the antioxidant and

antidiabetic properties of EGCG and we made an attempt to study the *in vitro* activity of EGCG given that polyphenols can stabilize excess free radicals, preventing the establishment of oxidative stress.

Therefore, our *in vitro* study have focused on evaluating antiradical and antioxidant activities (DPPH, FRAP, NO, and the total antioxidant capacity) of epigallocatechin–3-gallate (EGCG) shows the powerful antioxidant effect of this bioactive molecule. EGCG may interact directly with ROS or act indirectly by inhibiting ROS-generating enzymes or chelating potentially pro-oxidizing metal ions [20]. The antioxidant activity of EGCG was also confirmed by the trapping power of the DPPH radical [21]. Indeed, it is known that flavonoids are natural antioxidants that increase the expression of antioxidant enzymes (SOD, catalase and GSH-Px) at the transcriptional level [22]. Previous scientific studies have also demonstrated that the particular structure of polyphenols, whose flavonoids allow them to rapidly reduce superoxide radicals, peroxyl (ROO •), alkoxyl (RO •) and hydroxyl by hydrogen transfer. They also decrease the production of xanthine oxidase, a source of free radicals [23] (Table 1).

In the present study, alloxan treatment resulted in increase in blood glucose level of animals (Table 2). Both insulin and EGCG injections significantly reduced the blood glucose level.

On the other hand, the liver and kidney showed significant decrease in absolute weight and tissue damage in diabetic mice, whereas tissue regeneration and normal growth were found after treatment with EGCG. Such hepato and nephrocellular damage observed in diabetic mice likely is the result of oxidative stress [24]. Assessment of liver and kidney damage was done by measuring marker indices (*i.e.* ALT, AST, and LDH, Bilirubine, urea and creatinin), which leak into blood stream upon hepatic and kidney injuries [24–25]. In the present study, alloxan treatment caused severe liver and kidney damage, as evidenced by the significant increase in serum ALT, AST and LDH activities as well as elevated total and direct bilirubin levels in the plasma of diabetic mice. Urea and creatinine levels shows also a significant elevation in the diabetic mice.

Conversely, treatment with EGCG showed a significant decrease in serum AST, ALT and ALT, indicating a protective role for this molecule against liver dysfunctions (Table 3). Similar findings were reported earlier upon EGCG treatment against STZ-induced hepatotoxicity [26].

Diabetes mellitus may be accompanied by other metabolic perturbations such as those affecting lipid profiles; the most common are hypertriglyceridemia and hypercholesterolemia [27]. Accordingly, we observed a significant increase in TG, TC and LDL-Ch levels in alloxan-induced diabetic mice, suggesting lipid breakdown and mobilization of free fatty acids from the peripheral stores [27]. Similar observations were reported in diabetic patients where insulin deficiency caused a variety of perturbations in metabolic and regulatory processes, leading to the accumulation of total cholesterol and triglycerides. Conversely, we found that insulin injection decreased TG, TC and LDL-Ch levels, confirming the hypolipidemic effect of insulin. Similar observations could be done upon EGCG supplementation, reinforcing the notion that consumption of the catechins prevents hypercholesterolemia [28].

Oxidative stress has been known to induce the production of ROS scavenging enzymes [29]. These enzymes are supposed to respond in a compensatory response, increasing enzyme activity in diabetic mice, in an attempt to maintain homeostasis. Our findings on disturbed ROS enzymatic levels due to hyperglycaemia and their amelioration by plant extract are congruent with previous reports [30–31].

Indeed, a significant decrease in antioxidant enzymes activity in kidney and liver was found. This can be due to inhibition of the enzymes by increased production of free radicals during diabetes. SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O^{2-}), which damages the membrane and biological structures (Table 4–5). GPx and catalase activities in tissues of diabetic mice were decreased which could be due to the increased endogenous production of superoxide anions. H_2O_2, the substrate of GPx is itself toxic and can react with O_2^- in the presence of metal ions to form OH (Haber-Weiss reaction) and therefore results in higher levels of TBARS formation. High levels of unscavenged free radicals results in oxidative deterioration of polyunsaturated lipids leading to malondialdehyde formation.

Intraperitoneal administration of EGCG produces a statistically significant decrease in serum glucose concentration only in alloxan-induced diabetic fasted mice but not in healthy fasted mice. Therefore, the catechin did not affect insulin releasing from the pancreas of both animal groups. On the other hand, intraperitoneal administration of EGCG does not change serum glucose. Administration of EGCG results in the activation of SOD, CAT and GPx and therefore restoring near normal antioxidant enzyme levels. No histological changes was determined (Figure 2–3).

In conclusion, our results have shown that EGCG possess an hypoglycaemic effect on alloxan-induced hyperglycaemic mice intraperitoneally as well as being good inhibitors of the α-amylase an lipase enzymes. This study presented new findings about the biological and pharmacological potential of EGCG, contributing to the understanding of the action and efficacy of the use of this molecule in the digestive enzyme inhibitions and in antioxidant processe. Thus, these results open possibilities for further studies and controlled clinical trials using this catechin in order to be validated and used as an antidiabetic agent. This will be required to confirm its hypoglycaemic action and general safety.

Declarations

Acknowledgments

The present work was supported by the Laboratory of Animal Ecophysiology in the Faculty of Sciences of Sfax (UR/13 ES-73).The authors are grateful to Dr Xavier Dauvergne for his help in data analyses and to Naziha Khidiwi, translator and English teacher, for proofreading the manuscript.

Conflict of interest statement

The authors report no conflicts of interest associated with this manuscript.

Funding

This work has been funded by Ministry of Higher Education and Research, Scientific Research and Technology, Tunisia.

References

1. Bonnefont-Rousselot D. Glucose and reactive oxygen species. Curr Opin Clin Nutr Metab Care. 2002; 5: 561-8.
2. Masella R1, Di Benedetto R, Varì R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem. 2005; 16: 577-86. DOI: 10.1016/j.jnutbio.2005.05.013
3. Soussi A, Croute F, Soleilhavoup JP, Kammoun A, El-Feki A. Impact of green tea on oxidative stress induced by ammonium metavanadate exposure in male rats. C R Biol. 2006; 329:775-84. Epub 2006 Sep 20. DOI: 10.1016/j.crvi.2006.07.004
4. Ushio-Fukai M, Nakamura Y. Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. Cancer Lett. 2008; 266: 37-52. DOI: 10.1016/j.canlet.2008.02.044
5. Ahlem S, Khaled H, Wafa M, Sofiane B, Mohamed D, Jean-Claude M, Abdelfattah el F. Oral administration of Eucalyptus globulus extract reduces the alloxan-induced oxidative stress in rats. Chem Biol Interact. 2009; 181: 71-6. DOI: 10.1016/j.cbi.2009.06.006
6. Gupta MP, Solis NG, Esposito M, Sanchez S. Hypoglycemic activity of Neurolaena lobata (L), R. Br. J. Ethnopharmacol. 1984; 10: 323-7.
7. Koleva I, Van beek TA, Linssn JP, De groot A, Evstatieva LN. Screening of plant extracts of antioxydant activity: A compare study on three testing methods. Phytochem Anal. 2002; 13: 8-17.
8. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999 ; 269 : 337-341.
9. Megías C, Pastor-Cavada E, Torres-Fuentes C, GirónCalle J, Alaiz M, Juan R, et al. Chelating, antioxidant and antiproliferative activity of Vicia sativa polyphenol extracts. Eur Food Res Technol 2009; 230: 353-9.
10. Shirwaikar A, Prabhu KS, Punitha IS. In vitro antioxidant studies of Sphaeranthus indicus (Linn). Indian J Exp Biol. 2006; 44: 993-6.
11. Yagi KA. Simple Fluorometric assay for lipoperoxide in blood plasma. Biochem Med.1976; 15: 212–216.
12. Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105: 121–6.
13. Asada K, Takahashi M. Nagate M. Assay and inhibitors of spinach superoxide dismutase. Agr Biol Chem. 1974; 38: 471-473.
14. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Lab Clin Med. 1967; 70: 158-169.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem. 1951; 193: 265-271.
16. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys. 1959; 82: 70–77.
17. Vats V, Grover JK, Rathi SS. Evaluation of anti-hyperglycemic and hypoglycemic effect of Trigonella foenum-graecum Linn, Ocimum sanctum Linn and Pterocarpus marsupium Linn in normal and alloxanized diabetic rats. J Ethnopharmacol. 2002 ; 79, 95-100.
18. Ho HC, Huang CC, Lu YT, Yeh CM, Ho YT, Yang SF, Hsin CH, Lin CW Epigallocatechin-3-gallate inhibits migration of human nasopharyngeal carcinoma cells by repressing MMP-2 expression. J Cell Physiol. DOI: 10.1002/jcp.28696
19. Quezada-Fernández P, Trujillo-Quiros J, Pascoe-González S, Trujillo-Rangel WA, Cardona-Müller D, Ramos-Becerra CG, Barocio-Pantoja M, Rodríguez-de la Cerda M, Nérida Sánchez-Rodríguez E, Cardona-Muñóz EG, García-Benavides L, Grover-Páez F. Effect of green tea extract on arterial stiffness, lipid profile and sRAGE in patients with type 2 diabetes mellitus: a randomised, double-blind, placebo-controlled trial. Int J Food Sci Nutr. 2019; 1-9. DOI:10.1080/09637486.2019.1589430
20. Munin A, Edwards-Lévy F. Encapsulation of natural polyphenolic compounds ; a review. Pharmaceutics. 2011; 3: 793-829. DOI:10.3390/pharmaceutics3040793
21. Koch W, Kukula-Koch W, Głowniak K. Catechin Composition and Antioxidant Activity of Black Teas in Relation to Brewing Time. J AOAC Int. 2017 ; 100 : 1694-1699. DOI:10.5740/jaoacint.17-0235
22. Zakaria ZA, Mahmood ND, Omar MH, Taher M, Basir R. Methanol extract of Muntingia calabura leaves attenuates CCl4-induced liver injury: possible synergistic action of flavonoids and volatile bioactive compounds on endogenous defence system. Pharm Biol. 2019; 57: 335-344. DOI:10.1080/13880209.2019.1606836
23. Qin F, Yao L, Lu C, Li C, Zhou Y, Su C, Chen B, Shen Y. Phenolic composition, antioxidant and antibacterial properties, and in vitro anti-Hep G2 cell activities of wild apricot (Armeniaca Sibirica L. Lam) kernel skins. Food Chem Toxicol. 2019; 6: 354-364. DOI: 10.1016/j.fct.2019.05.007
24. Gargouri M, Magné C, El Feki A. Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation. Nutr Res. 2016 ; 36 : 1255-1268. DOI:10.1016/j.nutres.2016.09.011
25. Gargouri , Hamed H, Akrouti A, Dauvergne X, Magné C, El Feki A. Effects of Spirulina platensis on lipid peroxidation, antioxidant defenses, and tissue damage in kidney of alloxan-induced diabetic rats. Appl Physiol Nutr Metab. 2018 ; 43 : 345-354. DOI: 10.1139/apnm-2017-0461
26. Li X, Chen Y, Shen JZ, Pan Q, Yang W, Yan H, Liu H, Ai W, Liao W, Guo S Epigallocatechin Gallate Inhibits Hepatic Glucose Production in Primary Hepatocytes via Downregulating PKA Signaling Pathways and Transcriptional Factor FoxO1. J Agric Food Chem. 2019; **67**: 3651-3661. DOI:10.1021/acs.jafc.9b00395

27. Sefi M, Fetoui H, Soudani N, Chtourou Y, Makni M, Zeghal N. Artemisia campestris leaf extract alleviates early diabetic nephropathy in rats by inhibiting protein oxidation and nitric oxide end products. *Pathol Res Pract.* 2012 ; 208 : 157-62. DOI:10.1016/j.prp.2012.01.002

28. Soussi A, Abdennabi R, Ghorbel F, Murat JC, El Feki AF. Ameliorated Effects of (-)-Epigallocatechin Gallate Against Toxicity Induced by Vanadium in the Kidneys of Wistar Rats. Biol Trace Elem Res. 2017; 180: 239-245. DOI:10.1007/s12011-017-1004-4

29. Newsholme P, Cruzat VF, Keane KN, Carlessi R, Bittencourt PI. Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J. 2016; 473: 4527-4550. DOI:10.1042/BCJ20160503C

30. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol. 2002 ; 81 : 155-60.

31. Casanova E, Salvadó J, Crescenti A, Gibert-Ramos A. Epigallocatechin Gallate Modulates Muscle Homeostasis in Type 2 Diabetes and Obesity by Targeting Energetic and Redox Pathways: A Narrative Review. Int J Mol Sci. 2019 ; 27 : 20. DOI:10.3390/ijms20030532

Tables

Table 1– Antioxidant activities of EGCG

	Antioxidant activities			
	TAC[a]	DPPH[b]	FRAP[c]	NO• test[d]
EGCG (IC_{50})	811.74	4.64	0.645	0.12

Values represent the means of three replicates ± SE of six sera per group.
[a] Total antioxidant capacity (mg AAE g^{-1}DW)
[b] DPPH scavenging activity (µg mL^{-1})
[c] FRAP (µg mL^{-1})
[d] NO• test (mg/ml)

Table 2- Plasma glucose level (UI/l) in diabetic mice upon treatment with EGCG or insulin after 15 days of treatment.

	C	D	D+I	D+EGCG
Plasma glucose level	6.05 ± 0.02	$24.50 \pm 0.2^{***}$	$4.30 \pm 0.01^{+++}$	$7.60 \pm 0.01^{+++}$

C: Control; **D:** Diabetic; **(D+I):** Diabetic mice treated with insulin; **(D+ EGCG):** Diabetic mice injected by EGCG.
Each value represents the mean ± SE of six sera per group.
Comparison (**D**) *vs* control (**C**) group: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.
Comparison (**D+I**) or (**D+EGCG**) *vs* diabetic (**D**) group: $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$.

Table 3- Absolute (g) and relative (%) weights of mice liver and kidney after 15 days of treatment with EGCG or insulin.

Treatments	Absolute weight (g)	
	Liver	Kidney
C	1.61 ± 0.08	0.44 ± 0.02
D	$1.35 \pm 0.14^{*}$	0.44 ± 0.04
D+EGCG	1.68± 0.08	0.41 ± 0.01
D+I	1.66 ± 0.1	0.49 ± 0.02

	Relative weight (%)	
	Liver	Kidney
C	5.17 ± 0.27	1.40 ± 0.08
D	$4.55 \pm 0.31^{*}$	1.64 ± 0.14
D+EGCG	5.27 ± 0.26	1.28 ± 0.04
D+I	5.09 ± 0.33	1.52 ± 0.08

C: Control; **D:** Diabetic; **(D+E):** Diabetic mice injected by EGCG; **(D+I):** Diabetic mice treated with insulin.
Values are expressed as means ± SE of 6 samples per group.
One-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (FLSD) test as a post hoc test for comparison between groups:
Comparison between (**D**) vs control (**C**) group: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.
Comparison between (**D+EGCG**) group *vs* diabetic (**D**) group: $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$.
Comparison between (**D+I**) group *vs* diabetic (**D**) group: $^{@}p < 0.05$; $^{@@}p < 0.01$; $^{@@@}p < 0.001$.

Table 4 – Hepatic biomarkers (AST, ALT, LDH, and total bilirubin) levels in mice serum at 15 days of treatment with EGCG or insulin.

Treatment	AST[1] (U/L)	ALT[2] (U/L)	LDH[3] (U/L)	T-bilirubin[4] (U/L)	Urea[5] (U/L)	Creatinine[6] (U/L)
C	354.15±8.68	69.25±6.66	4017±5.30	2.5±0.28	6.25±0.02	14±0.57
D	839.85±14.40**	119.25±4.99**	4701±35.51**	4.25±0.20**	13.30±0.11**	36.5±0.86**
D+EGCG	527.45±9.67**, ++	78.05±4.76++	4077.5±39.60++	3±0.00	6.65±0.02++, **, @@	35.5±0.86**, @@
D+I	493.75±7.53@@	68.15±3.72@@	291.08 ±15.32@@@	3±0.00	6.15±0.02@@	28±0.00@@

C: Control; D: Diabetic; (D+EGCG): Diabetic mice injected by EGCG; (D+I): Diabetic mice treated with insulin.
[1] AST : Aspartate transaminase; [2] ALT : Alanine transaminase; [3] LDH : Lactate dehydrogenase; [4] T-bilirubin : Total bilirubin.
Values are expressed as means ± S.E of 6 samples per group.
One-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (FLSD) test as a post hoc test for comparison between groups:
Comparison between (D) *vs* control (C) group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
Comparison between (D+E) group *vs* diabetic (D) group: + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$.
Comparison between (D+I) group *vs* diabetic (D) group: @ $p < 0.05$; @@ $p < 0.01$; @@@ $p < 0.001$.

Table 5 – Plasmatic triglycerides, cholesterol and HDL-Ch levels (in mg/dL) in mice serum after 15 days of treatment with EGCG or insulin.

Treatment	[1] TG (UI/L)	[2]T-Ch (UI/L)	[3]HDL-Ch (UI/L)
C	3.71±0.02	4.0±0.05	2.92±0.02
D	3.09±0.03**	3.8±0.00*	2.26±0.01**
D+EGCG	3.83±0.04++, @@	3.55±0.02++, @@	2.83±0.01++
D+I	3.22±0.01+	3.85±0.02	2.75±0.01++

C: Control; D: Diabetic; (D+EGCG): Diabetic mice injected by EGCG; (D+I): Diabetic mice treated with insulin.
[1] TG: Triglycerides; [2] T-Ch: Total cholesterol; [3] HDL-Ch: High density lipoproteins of cholesterol.
Values are expressed as means ± S.E of 6 samples per group.
One-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (FLSD) test as a post hoc test for comparison between groups:
Comparison between (D) *vs* control (C) group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
Comparison between (D+E) group *vs* diabetic (D) group: + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$.
Comparison between (D+I) group *vs* diabetic (D) group: @ $p < 0.05$; @@ $p < 0.01$; @@@ $p < 0.001$.

Figures

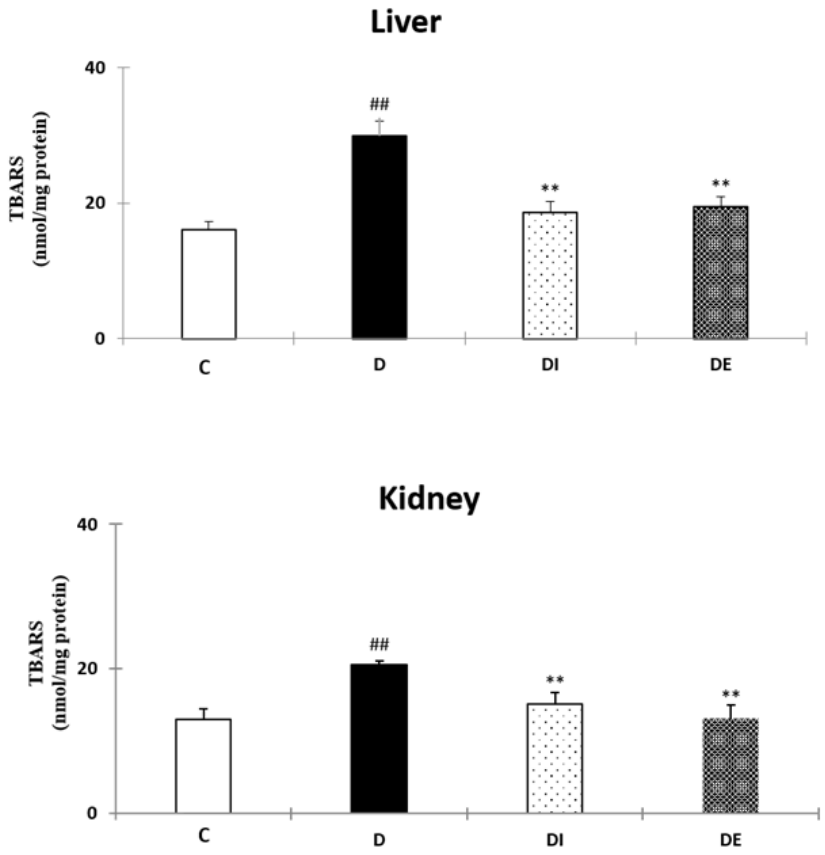

Figure 1

Effect of EGCG or insulin treatment (15 days) on oxidative status system activity in mouse liver and kidney.

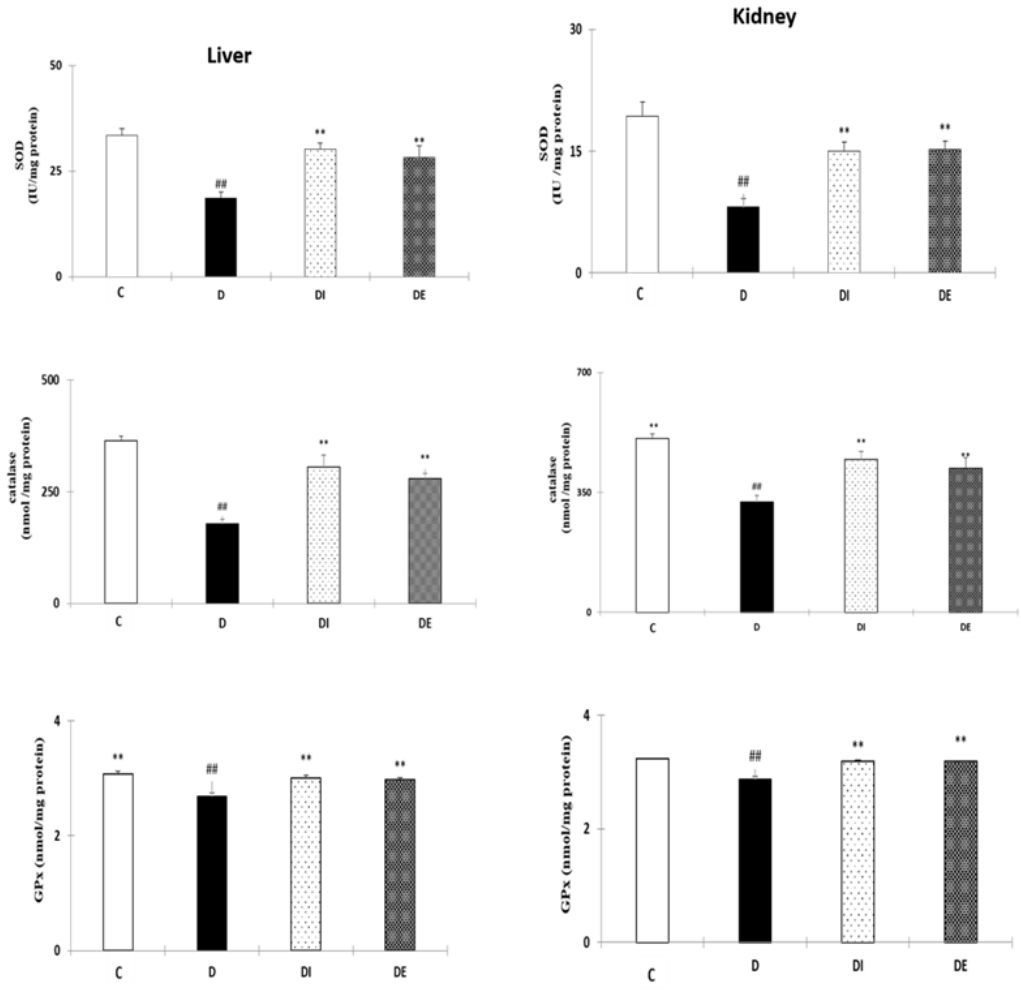

Figure 2

Effect of EGCG or insulin treatment (15 days) on Non-enzymatic antioxidant activities (SOD, Catalase, GPx) in mouse liver and kidney.

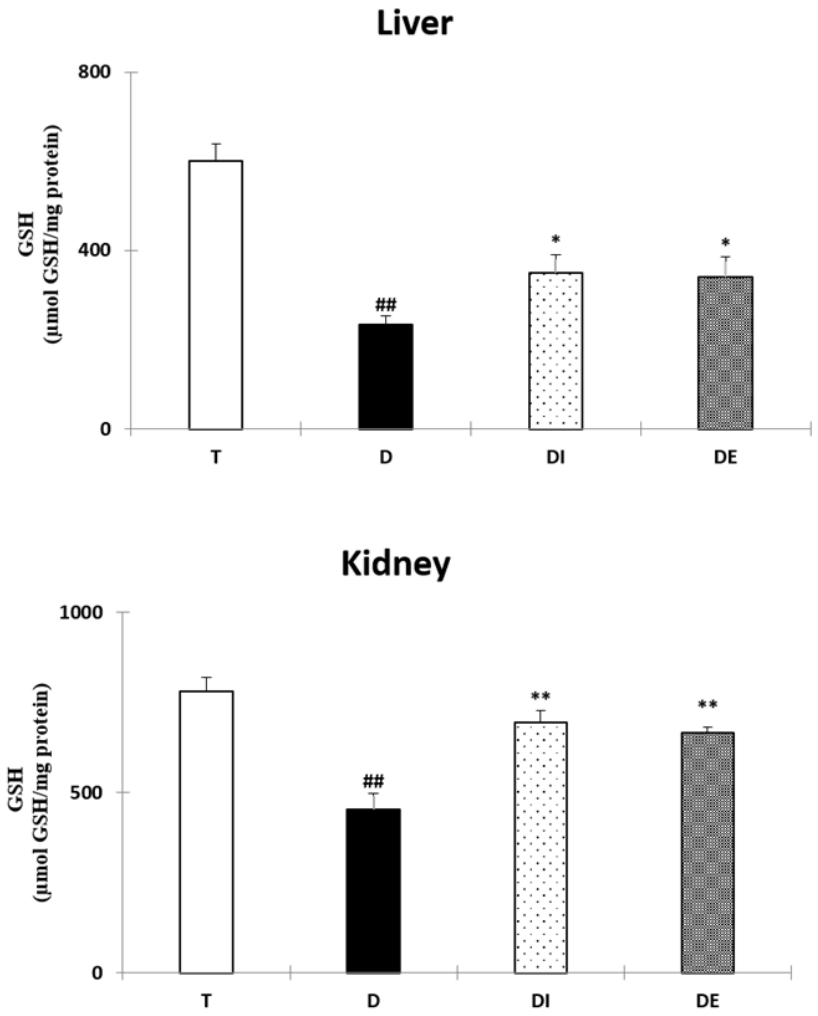

Figure 3

Effect of EGCG or insulin treatment (15 days) on GSH level in mouse liver and kidney.

Figure 4

Histological examinations of liver were conducted to understand the effect of different treatments on liver tissue histopathology. (A) control mice; (B) diabetic mice ; (C) diabetic mice treated with insulin; (D) diabetic mice treated with EGCG

Figure 5

Histological examinations of the renal tissues were conducted on all groups of mice to understand the effect of receiving treatments such as EGCG on renal histopathology induced by diabetes. Sections were stained with hematoxylin–eosin (magnification: 200x). (A') control mice; (B') diabetic mice; (C') diabetic mice treated with insulin; (D') diabetic mice treated with EGCG