Food & Function

Accepted Manuscript

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ROYAL SOCIETY OF CHEMISTRY

rsc.li/food-function

The protective role of virgin coconut oil on the alloxan-induced oxidative stress in liver, kidney and heart of diabetic rats

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View Article Online
DOI: 10.1039/C9FO00107G

Abstract

The aim of this study was to investigate the potential protective effect of virgin coconut oil (VCO) on oxidative stress parameters in the liver, kidneys and heart of alloxan-induced (150 mg/kg/i.p.) diabetes in rats. Our results showed that daily supplementation of VCO (20% of food) for 16 weeks significantly ($p<0.05$) ameliorates some deleterious effects caused by alloxan. The VCO reduced the diabetes-related increase in food (82.15±1.49 *vs* 145.51±4.81 g/kg b.m./day) and water (305.49±6.09 *vs* 583.98±14.80 mL/kg b.m./day) intake, and the decrease in the body mass gain (0.56±0.16 *vs* -2.13±0.49 g/100 g b.m./week). In all three tissues, diabetes caused an increase in total glutathione and sulfhydryl groups' concentration, and catalase and glutathione S-transferase activities, without changes in superoxide dismutase activity. Glutathione peroxidase activity was increased in kidney and heart, but not in the liver of the diabetic animals, while glutathione reductase activity was increased in the liver and the kidney, and not in the heart. The simultaneous VCO supplementation increased sulfhydryl group's concentration in all three tissues of diabetic animals and decreased glutathione S-transferase activity and glutathione concentration, without affecting glutathione reductase activity. In the liver of diabetic animals it decreased superoxide dismutase, catalase and glutathione peroxidase activities, in the heart catalase and glutathione peroxidase activities, and in the kidney catalase activity only. The results of Canonical Discriminant Analysis of oxidative stress parameters revealed that the VCO exerts its effects in a tissue-specific manner.

Keywords: alloxan; coconut oil; diabetes; oxidative stress; rats

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DOI: 10.1039/C9FO00107G

Introduction

The use of alloxan (ALX) is one of the most frequent approaches to diabetes induction in laboratory animals.[1] Alloxan causes rats' β-pancreatic cells destruction in dosage between 150 mg/kg and 200 mg/kg, with the intraperitoneal route of administration as the safest way to avoid toxic effects and to reduce overall mortality.[2] The reactive oxygen species (ROS) are increasingly formed in diabetes by glucose oxidation, non-enzymatic protein glycation and subsequent oxidative degradation of glycated proteins.[3] In order to protect macromolecules from the ROS adverse effects, cells developed defence system which includes enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione S-transferase (GST), and low-molecular-mass antioxidants such as glutathione (GSH) and free sulfhydryl groups (SH).[4] It was shown that cellular antioxidant status may be an important factor in the ethology of diabetes and that antioxidant treatment may reduce diabetic complications.[5]

Virgin coconut oil (VCO) is unrefined, cold pressed oil from a coconut palm (*Cocos nucifera* L.). It contains around 66% of carbohydrates (glucose, fructose, and sucrose), dietary fibres, phytosterols, polyphenols, tocopherols, and minerals.[6] Various studies have shown that VCO increases the serum level of high-density lipoprotein cholesterol and reduces the level of low-density lipoprotein cholesterol,[7] and exhibit strong antioxidant[8] and antidiabetic properties.[9]

Therefore, the aim of our study was to investigate the possible protective effects of VCO on oxidative stress biomarkers in the liver, kidney, and heart of alloxan-induced diabetic male Wistar albino rats. We investigated the SOD, CAT, GSH-Px, GR and GST activities, as well as the total GSH content, the SH group's concentration, and the level of lipid peroxidation (thiobarbituric acid reactive substances, TBARS). Ascorbic acid is not only a potent water-soluble antioxidant, but also an intravesicular store of reducing equivalents in adrenal chromaffin cells, so changes in the adrenal catecholamine turnover may affect the adrenal and serum vitamin C profile. Having that in mind, our experiments also included a study of total vitamin C (TVC), ascorbic acid (AA), and dehydroascorbic acid (DHA) levels in the adrenal gland, liver, and serum, as well as noradrenaline (NA) and adrenaline (AD) concentrations in adrenal glands.

Materials and methods

Animals and treatments

Male rats of Wistar strain (*Rattus norvegicus*) aged 3-3.5 months were used for the experiments. Animals were acclimated to 22±1°C and maintained under conditions of 12h

periods of light and dark, with free access to tap water and food. Rats were randomly divided into four groups, and housed in pairs in cages with six rats per group: Control (Con), Virgin Coconut Oil (VCO), Alloxan (ALX) and Alloxan+Virgin Coconut Oil (ALX+VCO) group. The experiment lasted for 16 weeks.

The Control group was fed on standard commercial rat food (Veterinary Institute, Subotica, Serbia). The single intraperitoneal (i.p.) injection of saline (5 mL/kg body mass) was administered on the first day of the experiment to allow comparability with other experimental groups (see below).

The Virgin Coconut Oil group was fed on standard commercial rat food enriched with coconut oil. VCO was added in the quantity to achieve the final concentration of around 20% percent of food and rigorously stirred for 5 minutes to allow an equal oil distribution. The food was prepared weekly: based on the measurements for 16 weeks, an average VCO concentration in food was 21.402±0.002%. In addition, the single i.p. injection of saline (5 mL/kg body mass) was administered on the first day of the experiment to allow comparability with other experimental groups (see below).

The Alloxan group was fed on standard commercial rat food, with the single i.p. injection of alloxan administered on the first day of the experiment. Alloxan was given in the dosage of 150 mg/kg body mass, dissolved in 5 mL of saline.

Alloxan+VCO group was fed in the same way as the VCO group and administered with the same alloxan treatment as the Alloxan group.

During the whole experiment, the body mass, body mass gain, food and water consumption, and fasting glycaemia were measured weekly. Glycaemia was measured after 16 hours of fasting, using the tail fresh capillary blood sample and handy Wellion CALLA Light blood glucose test strips system. Animal fasting started at the 4 p.m., which corresponds with the Zeitgeber time (ZT) 10, and the glycaemia measurement was performed at 8 a.m. next day, which corresponds with the ZT 2. Both time points were carefully chosen in respect to the rat's circadian rhythm. As the nocturnal animals, rats consume 80% of their daily food intake during the period between 5 p.m. and 6 a.m.[10] At the same time, at the ZT 2 rats exert very high efficacy in glycaemic regulation.[11]

All animal procedures were in compliance with the Serbian Law on animal welfare and were approved by the Veterinary Directorate of the Ministry of Agriculture, Forestry and Water Management, License number 323-07-10153/2016-05/1.

Sample preparation

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The animals were killed by decapitation using Harvard guillotine, and their blood was collected. The liver, kidneys, heart, and the adrenals of the rats were isolated and dissected out within 3 minutes, then placed in ice-cold 155 mmol NaCl and washed with the same solution.

Determination of oxidative stress biomarkers

The oxidative stress biomarkers were determined in the liver, kidney and heart samples. The tissues were minced and homogenized in 10 volumes of 25 mmol/L sucrose containing 10 mmol/L Tris-HCl, pH 7.5 at 1500 rpm using Janke & Kunkel (Staufen, Germany) IKA-Werk Ultra-Turrax homogenizer at 4°C (Rossi et al., 1983). Homogenates were then centrifuged at 4°C at 100000 x g for 90 minutes. The homogenates then were sonicated for 30s at 10 kHz on ice (Bandeline Sonopuls HD 2070), followed by centrifugation in a Beckman ultracentrifuge at 100000 × g for 90 min at 4˚C, and the obtained supernatants were used for biochemical analyses.

The activity of antioxidant defence enzymes was measured simultaneously in triplicate for each sample using a Shimadzu UV-1800 spectrophotometer and a temperature controlled cuvette holder. Total SOD activity was measured in the supernatant by the adrenaline method based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome.[12] SOD activity was expressed as U/g wet mass. CAT activity was determined as suggested by Beutler,[13] and expressed as μmol H_2O_2/min/g wet mass. The activity of GSH-Px was measured following the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate at 340 nm with t-butyl hydroperoxide,[14] and expressed in nmol NADPH/min/g wet mass. The activity of GR was evaluated as suggested by Glatzle et al.,[15] and expressed in nmol NADPH/min/g wet mass. GST activity toward 1-chloro-2,4-dinitro benzene (CDNB) as a substrate was assayed according to Habig et al.,[16] and expressed in nmol GSH/min/g wet mass. The GSH content was measured according to the method of Griffith,[17] based on the sequential oxidation of GSH by 5,5´-dithiobis 2-nitrobenzoicacid (DTNB) and further reduction by NADPH in the presence of GR. GSH content was expressed as μmol GSH /g tissue. The concentration of SH groups was determined using DTNB according to the Ellman method,[18] and expressed as nmol SH/g tissue. The concentration of lipid peroxides measured as thiobarbituric acid reactive substances (TBARS) in tissues of animals was assayed by the method of Rehncrona et al.,[19] using thiobarbituric acid (TBA) as a reagent. In this reaction, the coloured complex is formed and the absorbance was determined spectrophotometrically at 532 nm. Lipid peroxide concentration was expressed as nmol TBARS/g tissue. All the chemicals

used for oxidative stress parameters determination were SIGMA (St. Louis, MO, USA) products.

Determination of catecholamine concentration

The concentration of adrenaline (AD) and noradrenaline (NA) in the adrenal glands was performed according to the method of Stefanovic et al.[20] Ethylene glycol tetra-acetic acid (EGTA), 5-hydroxytryptamine (5-HT), DL-Noradrenaline hydrochloride (NA), perchloric acid (70%) and magnesium chloride ($MgCl_2x5H_2O$) were purchased from Sigma–Aldrich. Ammonium formate was supplied by Fisher Scientific, formic acid (49-51%) by Fluka, and methanol by J.T.Baker. Purified water was obtained via a BlueClearRO600P reverse osmosis water cleaner system with integrated BlueSoft07-MB mixed bed salt remover (Euro-Clear Ltd., Hungary).

Stock standard solutions of catecholamines [1mg/mL] were prepared in methanol and kept at -20˚C. Standard solutions were prepared by dilution of the stock standard solution in DEPROT (2% EGTA; 0.1 N $HClO_4$; 0.2% $MgCl_2$).

The ammonium formate buffer (100 mM, pH 3.6) was used as one of the mobile phase components. Ammonium formate (6.3 g) was accurately weighed, dissolved in approximately 800 mL of water and the pH was adjusted to the value of 3.6 with formic acid. The resulting solution was made up to 1 L with water.

Tissue samples were homogenized in DEPROT (1 mg:10 µL) using an Ultra-Turrax homogenizer, sonicated (3x10 sec) and centrifuged (30 min, 18000 rpm, 4°C). Supernatants were transferred in separate tubes and placed in the autosampler of the HPLC system.

Data were obtained using a Thermo Scientific (Dionex UltiMate 3000) HPLC system consisting of a degasser unit, binary pump, autosampler, column compartment and RS electrochemical detector equipped with the glassy carbon working electrode. A Hibar 125-4 LiCrospher100 RP-18 (5µm) HPLC column (Merck Millipore, Darmstadt, Germany) was used. Instrument control and data acquisition are carried out by the Chromeleon7 Chromatography Data System (Thermo Scientific).

The following chromatographic conditions were obtained after the method optimization. The mobile phase consisting of the ammonium formate buffer (100 mM, pH 3.6) as an A solution and methanol as a B solution was pumped at a flow rate of 500 µL/min with the following gradient. The run was started with a mobile phase consisting of 98% A and 2% B solution. Starting from 9th minute of run the part of B solution rose to reach 80% in the 13th minute. Starting from the 18th minute until the end of the run (25th min) the column was re-equilibrated with the initial mixture of mobile phase solutions (2% of A and 98% of B solution).

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DOI: 10.1039/C9FO00107G

The applied potential for electrochemical measurements was +850 mV and the separation temperature was set at 25 °C. The 40 µL of samples and standard solutions were applied into the system. The concentration of catecholamine was expressed as µg/mg tissue.

Determination of Vitamin C concentration

The concentration of total vitamin C (TVC), ascorbic acid (AA) and dehydroascorbic acid (DHA) in the liver, adrenals, and the serum of rats was performed according to the method of Nováková et al.[21] L-Ascorbic acid, phosphoric acid solution (49-51%), meta-phosphoric acid (MPA), sodium phosphate monobasic dihydrate and dithiothreitol (DTT) were purchased from Sigma–Aldrich. Purified water was obtained via a BlueClearRO600P reverse osmosis water cleaner system with integrated BlueSoft07-MB mixed bed salt remover (Euro-Clear Ltd., Hungary).

A stock standard solution of L-Ascorbic acid [1mg/mL] was prepared in 10% MPA and kept at -20˚C. Standard solutions were prepared by dilution of the stock standard solution in 10% MPA.

The phosphate buffer (160mM, pH 3.0) was used as the mobile phase. Sodium phosphate monobasic dihydrate (26 g) was accurately weighed, dissolved in approximately 800 mL of water and the pH was adjusted to a value of 3.0 with a phosphoric acid solution (49-51%). The resulting solution was made up to 1 L with water.

Tissue samples were homogenized in 10% MPA (1 mg:10 µL), using an Ultra-Turrax homogenizer, and sonicated (3×10 sec) while plasma samples were added to a 10% MPA (9:1, V:v). All the samples were centrifuged (30 min, 18000 rpm, 4°C) and supernatants were used for the analysis of native AA concentration. For the analysis of TVC concentration, 100µL of the above supernatant was treated with 300µL DTT solution (2.5mg/mL in phosphate buffer) for 30 min at 4˚C for complete conversion of DHA to AA. The mixture was then re-acidified with 200µL of 10% MPA and transferred to the autosampler unit.

Data were obtained using the same HPLC system as we described earlier. The following chromatographic conditions were obtained after the method optimization. The mobile phase consisting of the phosphate buffer (160mM, pH 3.0) was pumped over 10 min run in an isocratic flow of 800 µL/min. The applied potential for electrochemical measurements was +600 mV and the separation temperature was set at 25 °C. The 20 µL of samples and standard solutions were applied to the system. The concentration of vitamin C was expressed as µg/mg tissue and µg/ml serum.

Statistical analysis

The results are presented as the mean ± standard error. The level of statistical significance was defined as $p<0.05$. Data were checked for normality using Lilliefors and *Kolmogorov-Smirnov tests.* Differences in investigated parameters between the groups were calculated using One-way ANOVA. When significant differences were found, post hoc pair-wise comparisons were performed using Tukey HSD (honest significant difference) test. The existence and strength of the relationships between the examined parameters were determined by Canonical Discriminant Analysis (CDA) of all measured antioxidant components for all examined treatments.[22] All data were log transformed to improve normality of the variables. Statistical package STATISTICA 10.0 was used for all the analyses.

Results

The coconut oil supplementation causes the mild, although significant hypoglycaemic effect in VCO animals compared to the controls (Table 1). At the same time, alloxan treatment strongly increases the average fasting glycaemia in all diabetic animals compared to the controls. However, the hypoglycaemic effect of coconut oil seen in the VCO animals is missing in the diabetic animals since there is no significant difference in glycaemia between ALX and ALX+VCO groups.

Supplementation with coconut oil increases weekly body mass gain and lowers daily food and water intake in both intact and diabetic animals (Table 1). However, in comparison with the controls, diabetes by all means increases food and water intake, and decreases the body mass gain.

In all three tissues, diabetes does not change SOD activity in respect to controls, while it increases the CAT activity (Tables 2-4). In addition, it increases the GSH-Px activity in the kidney and heart, but not in the liver of the animals from ALX group. In intact and diabetic animals, VCO supplementation decreases activities of SOD, CAT and GSH-Px in the liver; CAT and GSH-Px activities in the heart, while in the kidney it decreases the CAT activity only. GST activity is increased in diabetes in all three tissues (Tables 2-4). In the liver and the heart supplementation with VCO lowers GST activity in both intact and diabetic animals in relation to their respective controls, while in the kidney it lowers GST activity in diabetic animals only.

GSH concentration simultaneously follows changes in GST activity (Tables 2-4). It is increased in diabetes in all examined tissues, decreased in the liver and the heart of intact and diabetic animals supplemented with the VCO, and decreased in the kidney of diabetic animals supplemented with VCO.

GR activity is increased in the liver and the kidney of animals from both diabetic groups, but not in the heart (Tables 2-4).

As a result of diabetes, SH group's concentration in ALX group is decreased in all three tissues. However, simultaneous supplementation with the coconut oil in ALX+VCO group of animals leads to their SH group's concentration recovery.

VCO supplementation lowers the level of lipid peroxidation in the liver and the heart of diabetic animals only, but not the kidney (Tables 2-4).

The results of Canonical Discriminant Analysis of oxidative stress parameters show clear separation between the liver, kidney and heart in all investigated groups of animals (Table 5, Figure 1). In the control group, CAT (0.712) and GSH (0.789) significantly influenced heterogeneity for Root 1, while GR (-1.097) did it for Root 2 (Table 5, Figure 1A). In animals administered with VCO, GR (-1,045) and GSH (1.244) mostly influenced differences in Root 1, whereas CAT (0.926) and GR (0.929) did the same in Root 2 (Table 5, Figure 1B). In ALX treated rats, CAT (2.828), GSH (2.423) and GST (1.186) significantly influenced differences in Root 1, while SOD (1.112) and GR (-1,345) influenced differences in Root 2 (Table 5, Figure 1C). In ALX+VCO animals, GSH-Px (1.165) and GSH (1.006) mostly influenced differences in Root 1, whereas GR (-0.753) did the same in Root 2 (Table 5, Figure 1D).

The serum TVC concentration is significantly higher in VCO group compared to the control (Figure 2). The reason of this is the increase of DHA fraction in serum, but not the AA. The possible source of the DHA increase in serum is not the liver, since in this tissue there are no significant differences in TVC, AA and DHA between VCO and control groups (Figure 3). However, in adrenal glands, coconut oil supplementation causes decrease in DHA fraction compared to the controls, but not in the AA (Figure 4), which implies the possible role of adrenals in the serum DHA rise (Figure 2).

In case of diabetes, the serum TVC concentration is significantly lower in ALX group compared to control, because of the decrease in the AA fraction in serum, and not the DHA (Figure 2). The possible reason of the serum TVC decrease in diabetes could be the liver, since in this tissue the concentrations of both AA and DHA are lower in the ALX group than in the controls (Figure 3).

While there are no significant changes in serum TVC and AA content between ALX and ALX+VCO groups, there is an increase in the serum DHA concentration in diabetic animals fed by coconut oil in respect to diabetic only animals (Figure 2). The possible reason for this could be the liver: it seems that coconut oil here stimulates ascorbate synthesis, since there is an increase in TVC, AA, and DHA content in ALX+VCO group compared to the ALX (Figure 3). This could be the sign of the protective VCO role in alloxan-induced diabetes: while alloxan treatment leads to the liver ascorbate synthesis decrease, adding coconut oil to the diet

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DOI: 10.1039/C9FO00107G

increases it. However, it cannot be excluded that the decrease in adrenal DHA content in ALX+VCO group compared to ALX (Figure 4) also contributes to the serum DHA concentration increase in ALX+VCO group in respect to the ALX (Figure 2).

As can be seen in Figure 5, there is a strong decrease in the adrenal noradrenaline content in VCO group compared to controls, and the same effect is present in ALX+VCO group compared to ALX. This decrease is partly the result of the improved conversion of noradrenaline to adrenaline, since the latter is significantly higher in ALX+VCO than in the ALX group.

Disscussion

Alloxan is a hydrophilic β-cytotoxin that induces chemical diabetes in a wide variety of animal species by damaging insulin-secreting cells.[23] Due to the structural similarity to glucose, it enters beta cells via GLUT2 transporters and induces glucokinase inhibition and ROS generation.[24] The ROS are generated through the redox cycle of alloxan GSH-mediated reduction into dialuric acid and further dialuric acid oxidation back to the alloxan. During this process, an alloxan radical is formed either by one-electron reduction of alloxan or by one-electron oxidation of dialuric acid, together with the superoxide anion radical, hydrogen peroxide, and hydroxyl radical.[25] Hydroxyl radicals are responsible for necrotic death of pancreatic cells, and as a result, alloxan induces a low blood insulin level and diabetes in animal models.[24]

As can be seen from our results, alloxan-induced diabetes rapidly increases fasting glycaemia, and food and water intake, and decreases body mass gain in both ALX and ALX+VCO groups compared to controls. At the same time, coconut oil supplementation lowers glycaemia, and food and water intake, and increases body mass gain, in the VCO group compared to controls. The part of this effect is also presented in the alloxan-treated animals: there is a significant decrease in food and water intake, and increase in body mass gain in the ALX+VCO group compared to ALX group. The reason why VCO increased body mass gain in both diabetic and non-diabetic animals, while lowering food and water intake, is that it is energetically highly efficient food, with a high content of fatty acids which can be metabolized into water (so-called metabolic water). As far as the hypoglycaemic effect of coconut oil is concerned, it is previously described in literature that lauric acid, whose content is especially high in VCO, has insulinotropic properties,[26,27] and that VCO polyphenols may enhance sensitivity to insulin and reduce insulin resistance.[28,29] Also, supplementation with VCO increases faecal abundance of probiotic bacteria, such as *Lactobacillus*, *Allobaculum* and *Bifidobacterium* species,[30] in a manner similar to that of the metformin.[31] Although it is not

clear how alterations in gut microbiota may promote beneficial effects in glucose homeostasis, a potential mechanism includes increased production of short-chain fatty acids.[32-34] However, the absence of the same effect in ALX+VCO group of animals can be explained by the fact that glycaemia is already extremely high there, so it cannot be further altered by mild hypoglycaemic effect of coconut oil.

Hyperglycaemia that accompanies diabetes is responsible for oxidative stress induction which leads to modifications of intracellular structures, proteins and DNA damage, with serious consequences on proper cell functioning.[35,36] There are numerous pathways by which hyperglycaemia increases oxidative stress. They include increased mitochondrial ROS production and the polyol pathway in which induction of aldose reductase and production of sorbitol cause oxidative stress that involves activation of NADPH and consequent ROS and reactive nitrogen species (RNS) generation. In the liver, the major source of ROS generation is a major cytochrome P450 isoform P4502E1 (CYP2E1). Induction of CYP2E1 by xenobiotics, drugs and pathophysiological conditions such as diabetes and alcoholism leads to increased oxidative stress.[37] Studies in humans and laboratory animals indicate that supplementation with antioxidants such as vitamin E and lipoic acid lessens the impact of oxidative damage caused by dysregulation of glucose metabolism in Type 2 diabetes.[38] In order to cope with the oxidative stress, tissues and cells are equipped with an antioxidative system, which includes enzymes such as SOD, CAT, GPX-Px, GR, and GST. Their role is to neutralize ROS thus maintaining their concentrations at a non-toxic, physiological level. However, when ROS generation exceeds a cell's antioxidant capacity it could result in oxidative stress.[39]

Our results showed that in all the examined tissues diabetes causes no significant changes in SOD activity compared to controls. So far, SODs are considered to be the only source of hydrogen peroxide production in cells.[40] From this point, unchanged SOD activity implies unchanged H_2O_2 formation, so it could be expected that hydrogen peroxidase activity should also stay unchanged. However, in our case, the CAT activity is increased in the liver, while in the kidneys and the heart both CAT and the GSH-Px activities are increased. A possible explanation of these changes could be the inhibition of SOD activity by a high concentration of H_2O_2. An enzyme can be inhibited by a product of its activity,[41] which has already been proven with SOD.[42,43] The fact that GSH-Px activity, while increased in the kidneys and the heart, remains unchanged in the liver, suggests that CAT activity in the liver is efficient enough to solely regulate H_2O_2 concentration, and that in the liver the main path of detoxification of peroxides is via CAT, while in the kidney and the heart is via both CAT and GSH-Px. This phenomenon is easy to explain: based on our data, CAT activity in the liver is

around 4 times higher than in the kidneys, and 40 times higher than in the heart. VCO supplementation decreases liver SOD activity in both normal and diabetic animals compared to their respective controls (i.e. Con and ALX groups), as well as CAT and GSH-Px activities. In the heart it also reduces CAT and GSH-Px activity in normal and diabetic animals, while in the kidney it reduces only CAT activity.

It is usually considered that the increase in SOD, CAT and GSH-Px activities is a sign of an increase of oxidative stress as well, bearing in mind the ROS-dependent mechanisms which regulate gene expression. They include activation of the redox-sensitive NF-κB nuclear factor, the principal transcription factor responsible for the upregulation of genes encoding manganese and copper zinc SODs, and the Nrf2 nuclear factor, the principal transcription factor responsible for the upregulation of genes encoding for several antioxidant enzymes, including CAT and GSH-Px.[44] In this respect, our data show that oxidative stress is increased in diabetes, and that coconut oil supplementation has an antioxidative effect, which is obviously tissue specific. Diabetes increases GST activity in all the examined tissues, while additional supplementation with coconut oil reduces it (in case of the liver and the heart it also reduces it in the VCO group alone). The same is true for GSH concentration, which simultaneously follows changes in the GST activity. Glutathione S-transferases comprise a family of phase II metabolic isozymes which catalyse the conjugation of the reduced form of glutathione to xenobiotic substrates for the purpose of detoxification. The activity of GSTs is dependent upon a steady supply of GSH, so it makes sense that increased GST activity will result in intracellular GSH content increase. Nevertheless, a similar trend between GST activity and GSH concentration is already described in literature.[45] Since the promoters of cytosolic and microsomal GSTs contain redox sensitive response elements through which they are transcriptionally activated during exposure to oxidative stress,[46] our results clearly show that diabetes causes oxidative stress and that VCO has a protective role.

SH group's concentration, which reflects the net result of GSH, GSH-Px, GST and GR interaction, basically represents reduced, thus antioxidative potential of a cell.[47] Alloxan treatment decreases SH group's concentration in all three tissues, indicating increased oxidative stress and reduced antioxidative capacity in diabetes. Simultaneous supplementation with coconut oil leads to the SH content recovery, which confirms protective effect of VCO in diabetes. GR activity, responsible to maintain all enzymatic and non-enzymatic SH groups in reduced state, is in the liver and kidney increased in both ALX and ALX+VCO groups. While GR activity increase in diabetes is in line with the previously described deleterious effects of diabetes, its elevation in the ALX+VCO group is unclear.

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DOI: 10.1039/C9FO00107G

Finally, the level of lipid peroxidation, which is considered to be a general indicator of oxidative stress, is decreased in the liver and the heart of animals from ALX+VCO group, compared to the diabetic group only. However, it is strange that diabetes alone does not change the TBAR level compared to controls.

In line with the literature data, our results proved the detrimental effect of diabetes on the antioxidative status in different tissues, and ameliorative effect of VCO.[48] However, our results also showed that individual enzyme activities cannot be taken as valid parameters in assessing some physiological conditions. All elements of antioxidative system act in a cooperative or synergistic manner to ensure global cell protection. In some cases, oxidative stress can cause a decrease in the activity of a particular enzyme, but in some other cases it can cause its increase. For this reason, we used canonical discriminant analysis in order to statistically analyse the oxidative stress parameters. CDA separates groups according to the complete organization of individual components, calculates the differences between groups according to the composition of their individual variables, calculates discriminant functions and identifies individual components which contribute to most of the differences. Our results show clear separation between tissues in all the investigated groups of animals. Comparing Con and ALX animals, diabetes causes differences between the kidneys and the heart *vs* the liver for Root 1, and between the kidneys and the heart for Root 2. In rats administered with VCO, Root 1 separates the liver and the kidneys, while Root 2 separates the liver and the kidneys *vs* the heart. In ALX+VCO co-treated animals, the liver was separated *vs* the heart for Root 1, while the liver and the heart were separated *vs* the kidneys for Root 2. From the overall CDA, it can be concluded that there is a similar scheme of distribution of investigated parameters in Con, ALX, and ALX+VCO groups of animals for both Root 1 and Root 2, while in the VCO group this scheme was changed due to differences between the kidneys and the heart.

The concentration of vitamin C in serum is the equilibrium of its synthesis, which in rats occurs in the liver,[49] and tissue consumption. Based on our results, the supplementation with VCO causes serum DHA fraction increase in both intact and diabetic animals. A possible source of this increase is not the liver, since in this tissue there are no significant differences in DHA concentration in VCO and ALX+VCO groups of animals versus controls. However, in the adrenal glands, coconut oil supplementation causes a decrease in DHA fraction compared to controls, but not in the AA, which implies a possible role of the adrenals in the serum DHA rise. As we published previously, the adrenals are able to selectively respond depending on the stressor: while the exposure of rats to environmental heat causes a significant decrease in the

adrenal vitamin C content accompanied by its increase in circulation, it is not the case with the exposure to cold.[50] The mechanisms which underlay this phenomenon are numerous. Vitamin C is thought to have a role in the regulation of corticosteroid synthesis in the adrenal glands by protection of the cytochrome P45011β from lipid peroxidation,[51] or by an increase of the adrenal capacity to convert cholesterol into pregnenolone.[52] Accordingly, adrenalectomy performed 24h before the exposure of rats to stress abolished the rise of AA in blood.[53] However, the role of vitamin C is not restricted to adrenal cortex only,[54] since ascorbic acid provides also an intravesicular store of reducing equivalents in the adrenal chromaffin cells.[55] The reducing equivalents serve for two purposes: the easily oxidized catecholamines must be kept in a reduced state, and the synthesis of noradrenaline by the dopamine beta-hydroxylase requires a reducing agent as a cofactor.[56] Hence, the changes in the adrenal catecholamine turnover may also affect the adrenal vitamin C profile. These results confirm that noradrenaline drop is not caused by its conversion to adrenaline, but rather by its release from the adrenals. If this is the case, then adrenal decrease in DHA content (as well as the accompanying DHA rise in the serum) could be the result of the increased noradrenaline release: during conversion of dopamine to noradrenaline ascorbic acid oxidize to DHA, and in DHA form it travels to blood together with noradrenaline.

In case of diabetes, the AA fraction in serum is significantly lower in ALX group compared to control. A possible reason to this decrease could be the liver: in this tissue the concentrations of both AA and DHA are lower in the ALX group than in the controls. It is known that diabetes causes a decrease in rat liver ascorbate synthesis, probably through the mechanism of impaired DHA to AA of recycling.[57] While there are no significant changes in serum AA content between ALX and ALX+VCO groups, there is an increase in the serum DHA concentration in diabetic animals fed by coconut oil in respect to the diabetic only animals. The possible reason of this could be the liver again, since there is an increase in the liver both of AA and DHA content in ALX+VCO group compared to the ALX. This could be a sign of the protective VCO role in alloxan-induced diabetes: while alloxan treatment leads to the liver ascorbate synthesis decrease, adding coconut oil to diet increases it.

According to the presented results, it can be concluded that in our experiments the administration of coconut oil reversed some toxic effects of alloxan induced diabetes. Also, our results confirmed that changes in antioxidant defence parameters in the liver, the kidneys and the heart are mainly tissue dependent. The liver proved to be the most important organ in the first line of defence against oxidative damage caused by alloxan, as well as the organ that first reacts to the protective effects of coconut oil.

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Acknowledgements — The present study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Nos. 173041 and 173023. This paper was proofread and edited by Professor Danka Sinadinovic, Faculty of Medicine, University of Belgrade (Department of Humanities).

Conflict of interest

There are no conflicts of interest to declare.

There is no conflict of interest, either financial or personal, or with other organizations.

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DOI: 10.1039/C9FO00107G

TABLES

Table 1. The data on the average weekly glycaemia (mmol/L), daily food (g/kg b.m.) and water (mL/kg b.m.) intake and body mass gain (g/100 g b.m.) in control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (VCO+ALX) treated rats during 16 weeks of experiment.

	Con	VCO	ALX	VCO+ALX
Glycaemia (mmol/L)	4.41±0.03	4.11±0.04^{a}	22.12±1.33^{ab}	24.18±1.35^{ab}
Food intake (g/kg b.m./day)	68.08±0.89	48.63±0.90^{a}	145.51±4.81^{ab}	82.15±1.49^{abc}
Water intake (mL/kg b.m./day)	95.83±1.89	68.48±1.57^{a}	583.98±14.80^{ab}	305.49±6.09^{abc}
Body mass gain (g/100 g b.m./week)	2.32±0.22	3.27±0.28^{a}	-2.13±0.49^{ab}	0.56±0.16^{abc}

Data are given as mean ± standard error. Minimal significant level: $p<0.05$.
Significantly different: ^{a} in respect to Con; ^{b} in respect to VCO; ^{c} in respect to ALX

Table 2. Activities of superoxide dismutase (SOD, U/g wet mass), catalase (CAT, μmol H_2O_2/min/g wet mass), glutathione peroxidase (GSH-Px, μmol NADPH/min/g wet mass), glutathione reductase (GR, nmol NADPH/min/g wet mass) and glutathione S-transferase (GST, nmol GSH/min/g wet mass), as well as concentrations of total glutathione (GSH, μmol/g tissue), sulfhydryl groups (SH, nmol/g tissue), lipid peroxides (TBARS, nmol/g tissue), and hydrogen peroxide (H_2O_2) in the liver of control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (VCO+ALX) treated rats.

	Con	VCO	ALX	VCO+ALX
SOD	7363.9±297.5	3181.5±186.8^{a}	8020.5±350.8	4290.0±321.9^{abc}
CAT	53876.2± 2116.0	36544.1±1349.7^{a}	69510.0±1228.8^{ab}	31675.5±2372.9^{ac}
GSH-Px	169.9±2.9	109.8±3.0^{a}	164.1±6.2^{b}	93.1±3.7^{abc}
GST	249892.4±7621.7	195364.6±3723.4^{a}	307594.4±8561.0^{ab}	206891.7±6723.4^{ac}
GSH	2411.8±69.6	1595.3±60.9^{a}	4298.1±245.6^{ab}	1439.8±87.1^{ac}
GR	7782.7±257.2	8373.8±407.0	11101.7±164.4^{ab}	10734.7±306.0^{ab}
SH	1098.7±11.3	1139.3±42.2	368.5±4.2^{ab}	1086.3±16.2^{c}
TBARS	0.99±0.07	0.99±0.08	0.93±0.04	0.70±0.04^{abc}

Data are given as mean ± standard error. Minimal significant level: $p<0.05$.
Significantly different: ^{a} in respect to Con; ^{b} in respect to VCO; ^{c} in respect to ALX

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Table 3. Activities of superoxide dismutase (SOD, U/g wet mass), catalase (CAT, µmol H_2O_2/min/g wet mass), glutathione peroxidase (GSH-Px, µmol NADPH/min/g wet mass), glutathione reductase (GR, nmol NADPH/min/g wet mass) and glutathione S-transferase (GST, nmol GSH/min/g wet mass), as well as concentrations of total glutathione (GSH, µmol/g tissue), sulfhydryl groups (SH, nmol/g tissue), lipid peroxides (TBARS, nmol/g tissue), and hydrogen peroxide (H_2O_2) in the kidney of control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (VCO+ALX) treated rats.

	Con	VCO	ALX	VCO +ALX
SOD	1144.9±27.5	1281.1± 40.8	1177.1±60.6	1228.6±42.0
CAT	12189.4±487.3	9298.7±353.0^{a}	13414.8±281.2^{ab}	7763.7±221.2^{abc}
GSH-Px	48.7±2.7	49.6±3.8	90.7±4.0^{ab}	88.3±2.6^{ab}
GST	28069.1±1314.6	27586.0±1184.6	80994.5±4190.1^{ab}	38945.0±2475.0^{abc}
GSH	23.7±0.8	20.2±0.6	74.6±3.7^{ab}	20.8±1.0^{c}
GR	14309.9±228.0	15076.6±194.8	16221.5±592.5^{a}	16261.3±257.0^{a}
SH	922.3±64.6	964.2±15.1	351.0±2.8^{ab}	973.0±51.4^{c}
TBARS	1.34±0.19	1.49±0.07	1.43±0.09	1.58±0.15

Data are given as mean ± standard error. Minimal significant level: $p<0.05$.
Significantly different: ^{a} in respect to Con; ^{b} in respect to VCO; ^{c} in respect to ALX

Table 4. Activities of superoxide dismutase (SOD, U/g wet mass), catalase (CAT, µmol H_2O_2/min/g wet mass), glutathione peroxidase (GSH-Px, µmol NADPH/min/g wet mass), glutathione reductase (GR, nmol NADPH/min/g wet mass) and glutathione S-transferase (GST, nmol GSH/min/g wet mass), as well as concentrations of total glutathione (GSH, µmol/g tissue), sulfhydryl groups (SH, nmol/g tissue), lipid peroxides (TBARS, nmol/g tissue), and hydrogen peroxide (H_2O_2) in the heart of control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil.

	Con	VCO	ALX	VCO +ALX
SOD	810.2±44.1	733.2±31.8	862.9±16.6	878.3±31.8
CAT	1039.4±87.2	565.4±91.8^{a}	2323.6±232.1^{ab}	972.6±130.7^{bc}
GSH-Px	35.2±1.4	17.5±0.5^{a}	48.2±1.3^{ab}	18.37±0.3^{ac}
GST	12343.5± 284.8	4932.8±280.1^{a}	13921.9±275.0^{ab}	6923.1±325.5^{abc}
GSH	856.4±27.2	587.5±20.7^{a}	1057.8±23.5^{ab}	412.8±30.0^{abc}
GR	1021.6±22.4	1104.1±21.9	1115.5±50.3	1152.0±45.3

SH	1071.9±9.6	1071.8±6.0	354.2±6.0[ab]	1064.0±2.7[c]
TBARS	0.82±0.07	0.91±0.08	0.87±0.04	0.38±0.03[abc]

Data are given as mean ± standard error. Minimal significant level: $p<0.05$.
Significantly different: [a] in respect to Con; [b] in respect to VCO; [c] in respect to ALX

Table 5. Canonical discriminant analysis of oxidative stress parameters in all investigated tissues on the factor plane in control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (VCO+ALX) treated rats.

	Con		**VCO**		**ALX**		**ALX+VCO**	
	Root 1	**Root 2**	**Root 1**	**Root 2**	**Root 1**	**Root 2**	**Root 1**	**Root 2**
SOD	0.363	-0.326	-0.230	0.504	-0.289	1.112*	0.574	-0.022
CAT	0.712*	0.216	-0.176	0.926*	2.828*	-0.116	0.187	0.240
GPX	0.651	-0.240	0.198	-0.123	-0.529	0.879	1.165*	-0.376
GR	-0.086	-1.097*	-1.045*	0.929*	0.770	-1.345*	0.764	-0.753*
GSH	0.789*	0.257	1.244*	-0.719	2.423*	-0.145	1.006*	0.660
GST	0.593	-0.038	0.641	0.367	1.186*	-0.542	0.775	0.679
SH	0.667	0.089	0.735	-0.352	0.413	-0.225	-0.315	0.049
Eigenval	592.380	132.501	383.927	209.060	5391.392	99.695	625.923	173.523
Cum. Prop	0.817	1.000	0.647	1.000	0.982	1.000	0.783	1.000

The sign * marks parameters that statistically significantly contribute to separation by root 1 or 2

Figure 1. Canonical discriminant analysis of oxidative stress parameters in all investigated tissues on the factor plane in control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (VCO+ALX) treated rats.

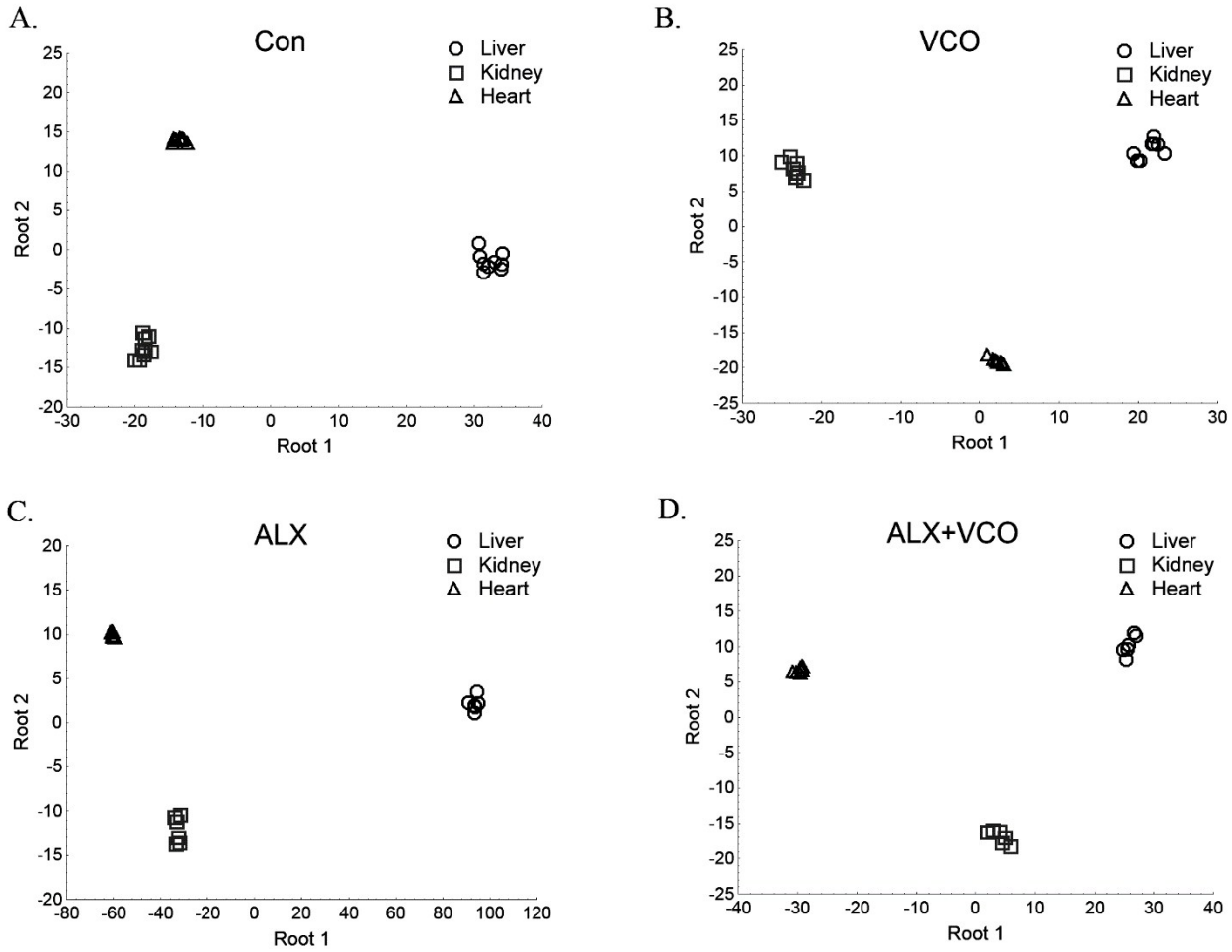


Figure 2. The concentrations of total vitamin C (TVC), ascorbic acid (AA) and dehydroascorbic acid (DHA) in the serum of control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (ALX+VCO) treated rats. Data are given as mean ± standard error. Minimal significant level: $p<0.05$. Significantly different: **a** in respect to Con; **b** in respect to VCO; **c** in respect to ALX.

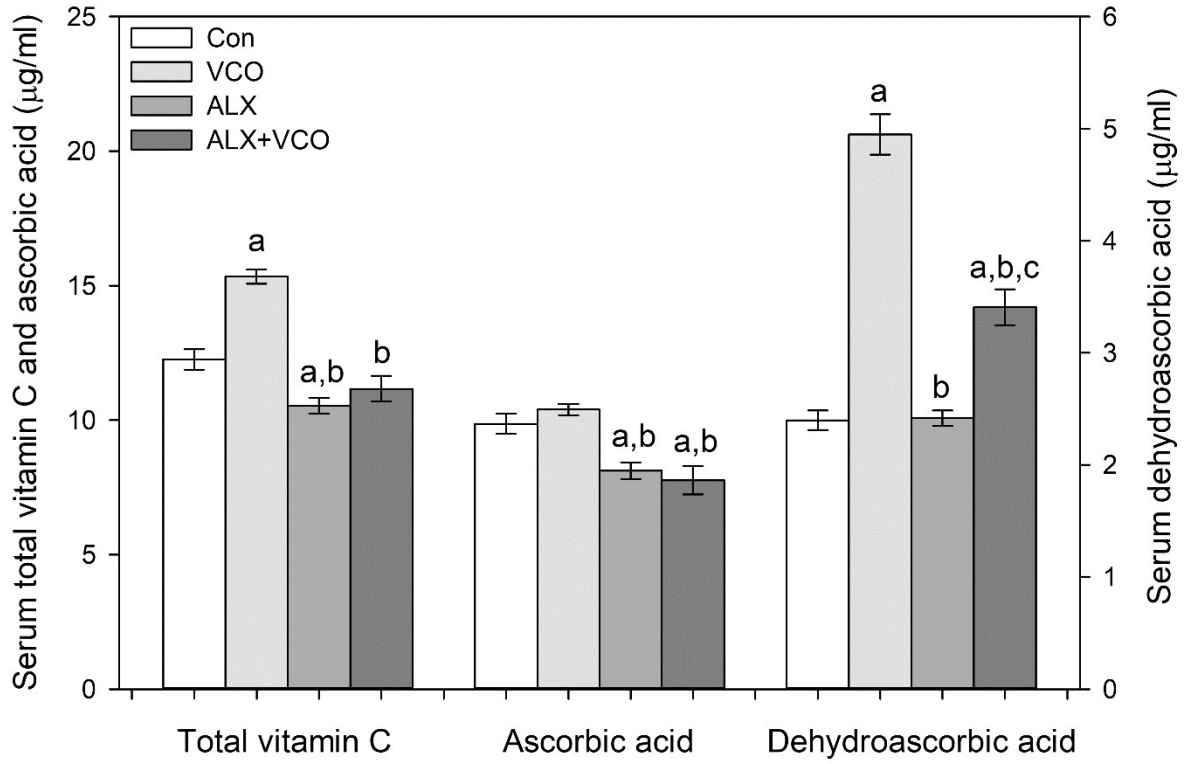


Figure 3. The concentrations of total vitamin C (TVC), ascorbic acid (AA) and dehydroascorbic acid (DHA) in the liver of control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (ALX+VCO) treated rats. Data are given as mean ± standard error. Minimal significant level: $p<0.05$. Significantly different: **a** in respect to Con; **b** in respect to VCO; **c** in respect to ALX.

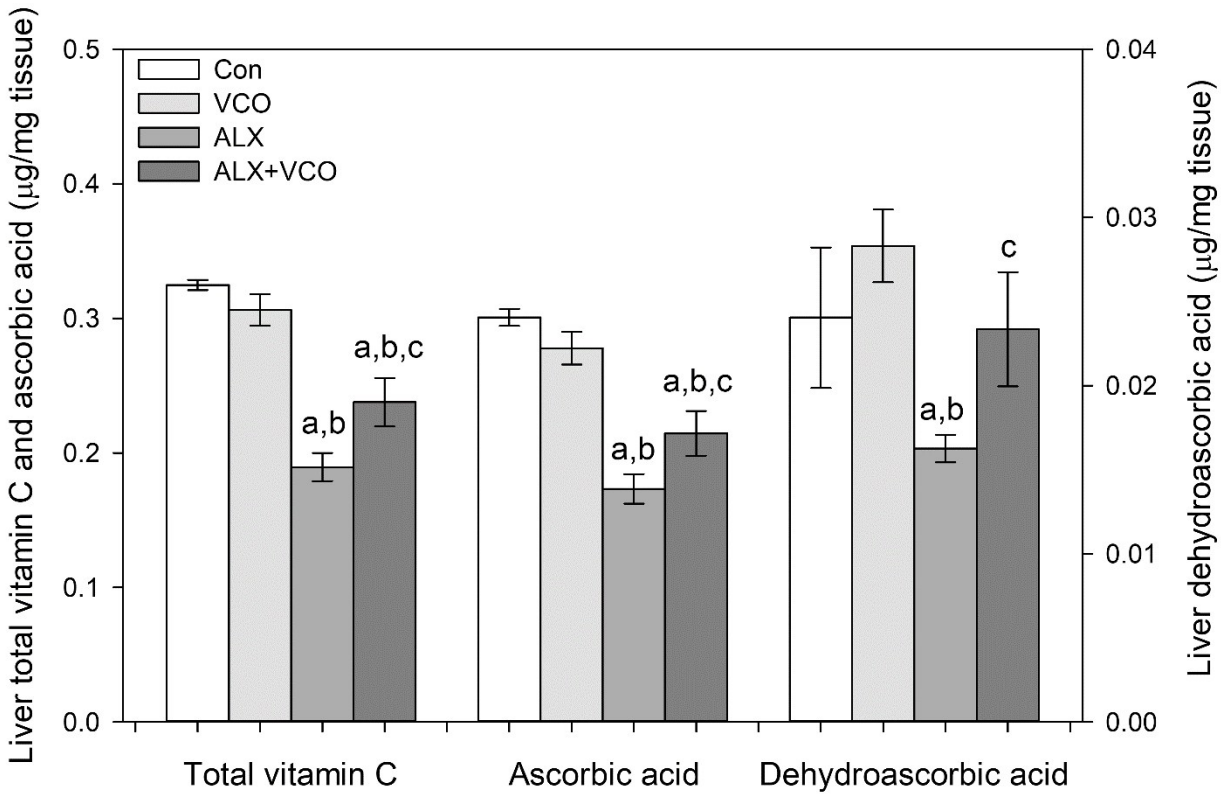


Figure 4. The concentrations of total vitamin C (TVC), ascorbic acid (AA) and dehydroascorbic acid (DHA) in the adrenal glands of control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (ALX+VCO) treated rats. Data are given as mean ± standard error. Minimal significant level: $p<0.05$. Significantly different: **a** in respect to Con; **b** in respect to VCO; **c** in respect to ALX.

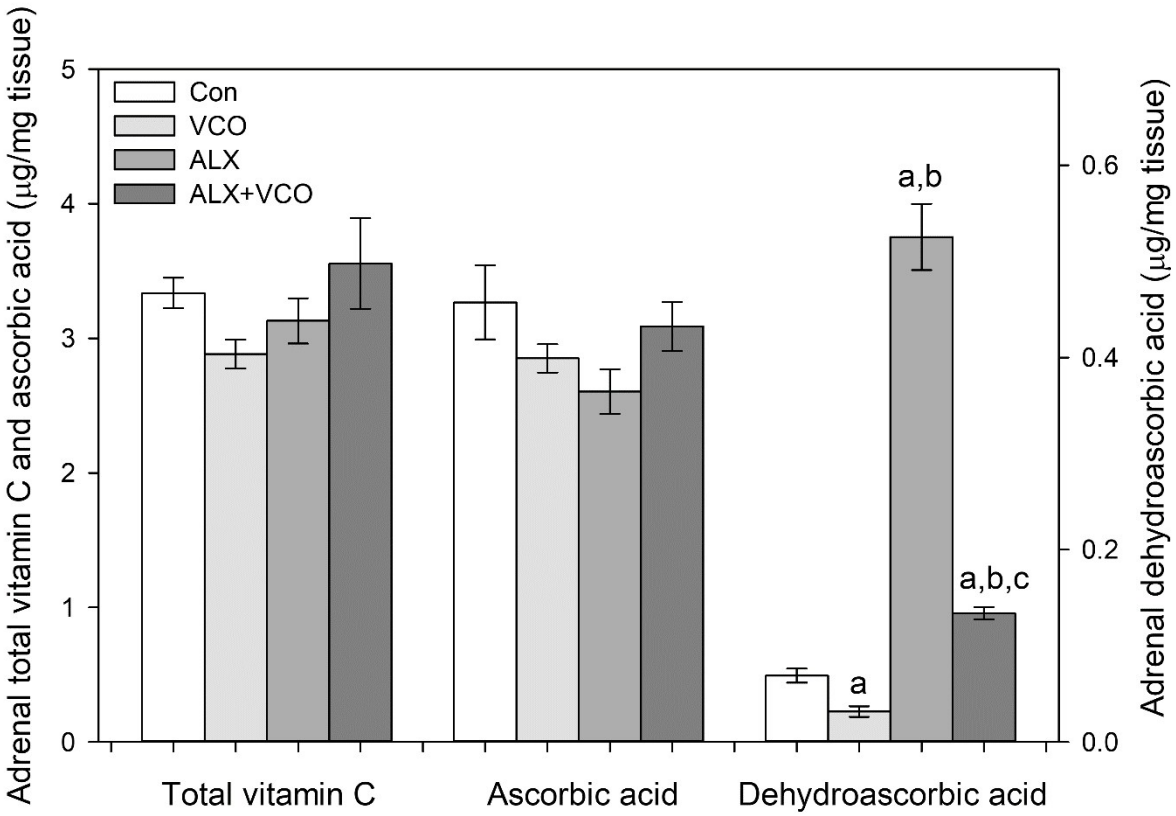
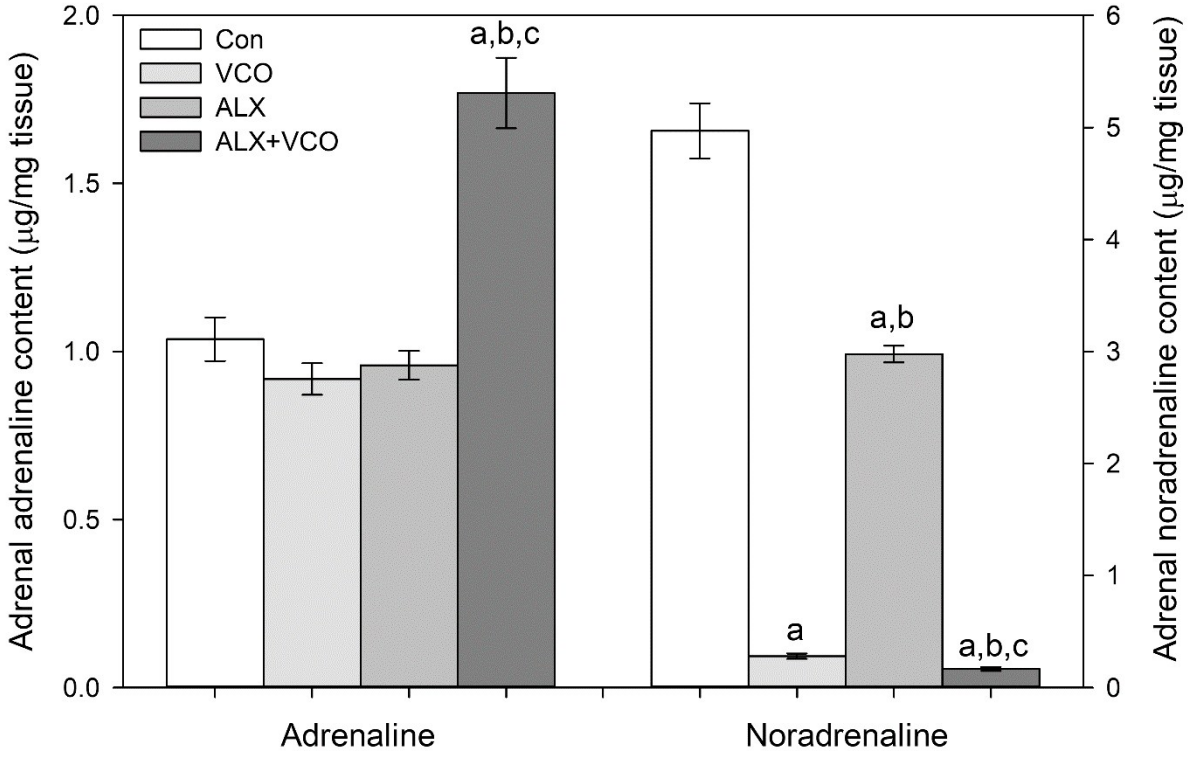


Figure 5. The concentrations of adrenaline (AD) and noradrenaline (NA) in the adrenal glands of control (Con), virgin coconut oil (VCO), alloxan (ALX) and alloxan+virgin coconut oil (ALX+VCO) treated rats. Data are given as mean ± standard error. Minimal significant level: $p<0.05$. Significantly different: **a** in respect to Con; **b** in respect to VCO; **c** in respect to ALX.



References

1. M. Radenkovic, M. Stojanovic and M. Prostran, Experimental diabetes induced by alloxan and streptozotocin: The current state of the art, *J. Pharmacol. Toxicol. Methods*, 2016, **78**, 13-31.
2. I. F. Federiuk, H. M. Casey, M. J. Quinn, M. D. Wood and W. K. Ward, Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment, *Comp. Med.*, 2004, **54**, 252-257.
3. M. Gargouri, C. Magne and A. El Feki, Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation, *Nutr. Res.*, 2016, **36**, 1255-1268.
4. B. Halliwell and J. M. C. Gutteridge, *Free radicals in biology and medicine*, Oxford University Press, Oxford, United Kingdom ;, Fifth edition. edn., 2015.
5. I. Dahech, K. S. Belghith, K. Hamden, A. Feki, H. Belghith and H. Mejdoub, Oral administration of levan polysaccharide reduces the alloxan-induced oxidative stress in rats, *Int J Biol Macromol*, 2011, **49**, 942-947.
6. A. Manivannan, R. Bhardwaj, S. Padmanabhan, P. Suneja, K. B. Hebbar and S. R. Kanade, Biochemical and nutritional characterization of coconut (Cocos nucifera L.) haustorium, *Food Chem.*, 2018, **238**, 153-159.
7. C. Scaccini, M. Nardini, M. D'Aquino, V. Gentili, M. Di Felice and G. Tomassi, Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions, *J Lipid Res*, 1992, **33**, 627-633.
8. K. G. Nevin and T. Rajamohan, Virgin coconut oil supplemented diet increases the antioxidant status in rats, *Food Chem.*, 2006, **99**, 260-266.
9. M. DebMandal and S. Mandal, Coconut (Cocos nucifera L.: Arecaceae): in health promotion and disease prevention, *Asian Pac. J. Trop. Med.*, 2011, **4**, 241-247.
10. J. Sidlo, M. Zaviacic and P. Kvasnicka, Night and day differences in the food-intake of laboratory rats Wistar and Koletsky strains, *Bratisl. Lek. Listy*, 1995, **96**, 655-657.
11. S. E. la Fleur, A. Kalsbeek, J. Wortel, M. L. Fekkes and R. M. Buijs, A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus, *Diabetes*, 2001, **50**, 1237-1243.
12. H. P. Misra and I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, *J Biol Chem*, 1972, **247**, 3170-3175.
13. E. Beutler, *Red cell metabolism : a manual of biochemical methods*, Grune & Stratton, Orlando, FL, 3rd edn., 1984.
14. M. Tamura, N. Oshino and B. Chance, Some characteristics of hydrogen- and alkylhydroperoxides metabolizing systems in cardiac tissue, *J. Biochem.*, 1982, **92**, 1019-1031.
15. D. Glatzle, J. P. Vuilleumier, F. Weber and K. Decker, Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans, *Experientia*, 1974, **30**, 665-667.
16. W. H. Habig, M. J. Pabst and W. B. Jakoby, Glutathione S-transferases. The first enzymatic step in mercapturic acid formation, *J Biol Chem*, 1974, **249**, 7130-7139.
17. O. W. Griffith, Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine, *Anal Biochem*, 1980, **106**, 207-212.
18. G. L. Ellman, Tissue sulfhydryl groups, *Arch Biochem Biophys*, 1959, **82**, 70-77.
19. S. Rehncrona, D. S. Smith, B. Akesson, E. Westerberg and B. K. Siesjo, Peroxidative changes in brain cortical fatty acids and phospholipids, as characterized during Fe2+-

and ascorbic acid-stimulated lipid peroxidation in vitro, *J Neurochem*, 1980, **34**, 1630-1638.

20. B. Stefanovic, N. Spasojevic, P. Jovanovic, N. Jasnic, J. Djordjevic and S. Dronjak, Melatonin mediated antidepressant-like effect in the hippocampus of chronic stress-induced depression rats: Regulating vesicular monoamine transporter 2 and monoamine oxidase A levels, *Eur. Neuropsychopharmacol.*, 2016, **26**, 1629-1637.
21. L. Nováková, P. Solich and D. Solichová, HPLC methods for simultaneous determination of ascorbic and dehydroascorbic acids, *TrAC Trends in Analytical Chemistry*, 2008, **27**, 942-958.
22. R. B. Darlington, S. L. Weinberg and H. J. Walberg, Canonical Variate Analysis and Related Techniques, *Review of Educational Research*, 1973, **43**, 433-454.
23. S. H. Lee, J. S. Han, S. J. Heo, J. Y. Hwang and Y. J. Jeon, Protective effects of dieckol isolated from Ecklonia cava against high glucose-induced oxidative stress in human umbilical vein endothelial cells, *Toxicol In Vitro*, 2010, **24**, 375-381.
24. S. Lenzen, The mechanisms of alloxan- and streptozotocin-induced diabetes, *Diabetologia*, 2008, **51**, 216-226.
25. J. A. Rosso, M. A. Astorga, D. O. Martire and M. C. Gonzalez, Alloxan-dialuric acid cycling: a complex redox mechanism, *Free Radic. Res.*, 2009, **43**, 93-99.
26. M. Garfinkel, S. Lee, E. C. Opara and O. E. Akwari, Insulinotropic potency of lauric acid: a metabolic rationale for medium chain fatty acids (MCF) in TPN formulation, *J. Surg. Res.*, 1992, **52**, 328-333.
27. B. Iranloye, G. Oludare and M. Olubiyi, Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats, *Journal of Diabetes Mellitus*, 2013, **3**, 221-226.
28. M. Siddalingaswamy, A. Rayaorth and F. Khanum, Anti-diabetic effects of cold and hot extracted virgin coconut oil, *Journal of Diabetes Mellitus*, 2011, **1**, 118-123.
29. A. Narayanankutty, R. K. Mukesh, S. K. Ayoob, S. K. Ramavarma, I. M. Suseela, J. J. Manalil, B. T. Kuzhivelil and A. C. Raghavamenon, Virgin coconut oil maintains redox status and improves glycemic conditions in high fructose fed rats, *J. Food Sci. Technol.*, 2016, **53**, 895-901.
30. S. Djurasevic, S. Bojic, B. Nikolic, I. Dimkic, Z. Todorovic, J. Djordjevic and D. Mitic-Culafic, Beneficial Effect of Virgin Coconut Oil on Alloxan-Induced Diabetes and Microbiota Composition in Rats, *Plant Foods Hum. Nutr.*, 2018, **73**, 295-301.
31. H. Wu, E. Esteve, V. Tremaroli, M. T. Khan, R. Caesar, L. Manneras-Holm, M. Stahlman, L. M. Olsson, M. Serino, M. Planas-Felix, G. Xifra, J. M. Mercader, D. Torrents, R. Burcelin, W. Ricart, R. Perkins, J. M. Fernandez-Real and F. Backhed, Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug, *Nat. Med.*, 2017, **23**, 850-858.
32. A. Koh, F. De Vadder, P. Kovatcheva-Datchary and F. Backhed, From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites, *Cell*, 2016, **165**, 1332-1345.
33. F. De Vadder, P. Kovatcheva-Datchary, C. Zitoun, A. Duchampt, F. Backhed and G. Mithieux, Microbiota-Produced Succinate Improves Glucose Homeostasis via Intestinal Gluconeogenesis, *Cell Metab.*, 2016, **24**, 151-157.
34. J. M. Wong, R. de Souza, C. W. Kendall, A. Emam and D. J. Jenkins, Colonic health: fermentation and short chain fatty acids, *J. Clin. Gastroenterol.*, 2006, **40**, 235-243.
35. A. C. Maritim, R. A. Sanders and J. B. Watkins, 3rd, Diabetes, oxidative stress, and antioxidants: a review, *J Biochem Mol Toxicol*, 2003, **17**, 24-38.

36. J. L. Martindale and N. J. Holbrook, Cellular response to oxidative stress: signaling for suicide and survival, *J Cell Physiol*, 2002, **192**, 1-15.
37. K. Chandrasekaran, K. Swaminathan, S. M. Kumar, D. L. Clemens and A. Dey, Increased oxidative stress and toxicity in ADH and CYP2E1 overexpressing human hepatoma VL-17A cells exposed to high glucose, *Integr. Biol. (Camb.)*, 2012, **4**, 550-563.
38. A. Dey and J. Lakshmanan, The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress and injury in liver, *Food Funct.*, 2013, **4**, 1148-1184.
39. D. Ahmed, V. Kumar, A. Verma, P. S. Gupta, H. Kumar, V. Dhingra, V. Mishra and M. Sharma, Antidiabetic, renal/hepatic/pancreas/cardiac protective and antioxidant potential of methanol/dichloromethane extract of Albizzia Lebbeck Benth. stem bark (ALEx) on streptozotocin induced diabetic rats, *BMC Complement. Altern. Med.*, 2014, **14**, 243.
40. C. Lennicke, J. Rahn, R. Lichtenfels, L. A. Wessjohann and B. Seliger, Hydrogen peroxide - production, fate and role in redox signaling of tumor cells, *Cell Commun Signal*, 2015, **13**, 39.
41. A. Cornish-Bowden, The origins of enzyme kinetics, *FEBS Lett.*, 2013, **587**, 2725-2730.
42. L. M. Casano, L. D. Gomez, H. R. Lascano, C. A. Gonzalez and V. S. Trippi, Inactivation and degradation of CuZn-SOD by active oxygen species in wheat chloroplasts exposed to photooxidative stress, *Plant Cell Physiol*, 1997, **38**, 433-440.
43. R. H. Gottfredsen, U. G. Larsen, J. J. Enghild and S. V. Petersen, Hydrogen peroxide induce modifications of human extracellular superoxide dismutase that results in enzyme inhibition, *Redox Biol*, 2013, **1**, 24-31.
44. L. Miao and D. K. St Clair, Regulation of superoxide dismutase genes: implications in disease, *Free Radic. Biol. Med.*, 2009, **47**, 344-356.
45. M. Sarhan and A. Al-Qahtani, The Distribution of Glutathione and Glutathione S-transferase Activity in the Organs of Dhub (The Agamid Lizard; Uromastyx aegyptius), *J. Biol. Sci.*, 2007, **7**, 558-561.
46. J. D. Hayes, J. U. Flanagan and I. R. Jowsey, Glutathione transferases, *Annu. Rev. Pharmacol. Toxicol.*, 2005, **45**, 51-88.
47. G. J. McBean, Cysteine, Glutathione, and Thiol Redox Balance in Astrocytes, *Antioxidants (Basel)*, 2017, **6**.
48. S. Arunima and T. Rajamohan, Effect of virgin coconut oil enriched diet on the antioxidant status and paraoxonase 1 activity in ameliorating the oxidative stress in rats - a comparative study, *Food Funct.*, 2013, **4**, 1402-1409.
49. A. P. Grollman and A. L. Lehninger, Enzymic synthesis of L-ascorbic acid in different animal species, *Arch Biochem Biophys*, 1957, **69**, 458-467.
50. J. Djordjevic, S. Djurašević, T. Vučković, N. Jasnić and G. Cvijić, Effect of cold and heat stress on rat adrenal, serum and liver ascorbic acid concentration, *Arch. Biol. Sci., Belgrade*, 2006, **58**, 161-164.
51. F. M. Finn and P. A. Johns, Ascorbic acid transport by isolated bovine adrenal cortical cells, *Endocrinology*, 1980, **106**, 811-817.
52. N. L. Doulas, A. Constantopoulos and B. Litsios, Effect of ascorbic acid on guinea pig adrenal adenylate cyclase activity and plasma cortisol, *J Nutr*, 1987, **117**, 1108-1114.
53. J. Y. Choi, I. C. d. Peña, S. Y. Yoon, T. S. Woo, Y. J. Choi, C. Y. Shin, J. H. Ryu, Y. S. Lee, G. Y. Yu and J. H. Cheong, Is the anti-stress effect of vitamin C related to adrenal gland function in rat?, *Food Sci. Biotechnol.*, 2011, **20**, 429–435.

54. P. Patak, H. S. Willenberg and S. R. Bornstein, Vitamin C is an important cofactor for both adrenal cortex and adrenal medulla, *Endocr. Res.*, 2004, **30**, 871-875.
55. D. Njus, J. Knoth, C. Cook and P. M. Kelly, Electron transfer across the chromaffin granule membrane, *J Biol Chem*, 1983, **258**, 27-30.
56. S. R. Bornstein, M. Yoshida-Hiroi, S. Sotiriou, M. Levine, H. G. Hartwig, R. L. Nussbaum and G. Eisenhofer, Impaired adrenal catecholamine system function in mice with deficiency of the ascorbic acid transporter (SVCT2), *FASEB J.*, 2003, **17**, 1928-1930.
57. M. Kashiba, J. Oka, R. Ichikawa, E. Kasahara, T. Inayama, A. Kageyama, H. Kageyama, T. Osaka, K. Umegaki, A. Matsumoto, T. Ishikawa, M. Nishikimi, M. Inoue and S. Inoue, Impaired ascorbic acid metabolism in streptozotocin-induced diabetic rats, *Free Radic. Biol. Med.*, 2002, **33**, 1221-1230.

Graphical abstract

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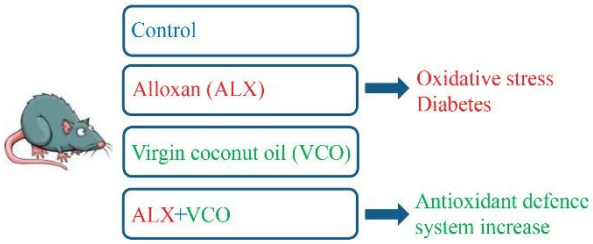
DOI: 10.1039/C9FO00107G



Our results show that VCO supplementation ameliorates some toxic effects of alloxan induced diabetes, and that changes are tissue specific.