Modulation of antioxidant status in streptozotocin−induced diabetic male wistar rats following intake of red palm oil and/or rooibos

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ARTICLE INFO

ABSTRACT

Objective: To investigate the role of red palm oil (RPO), rooibos tea extract (RTE) and their combined treatment (RPO + RTE) on antioxidant status in streptozotocin (STZ)−induced diabetic rats. **Methods:** Diabetes mellitus was induced by a single administration of streptozotocin (50 mg/kg) and the rats were treated for 7 weeks. Antioxidant enzymes [catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD)], antioxidant capacity [trolox equivalence antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC)] as well as total protein, albumin, globulin, total glutathione, conjugated diene and thiobarbituric acid reactive substances (TBARS) were investigated. **Results:** Treatment with RPO, RTE and RPO + RTE significantly (p>0.05) improved liver SOD and plasma ORAC in the diabetic rats. Similarly, diabetic rats treated with RTE and RPO + RTE enhanced liver GPx. A significant ($P<0.05$) increase in the plasma TBARS in the diabetic control group was observed when compared with the normal control group. Treatment of diabetic rats with RTE and RPO + RTE reduced plasma TBARS to a level not significantly different at $P<0.05$ from the normal control group. **Conclusions:** The results revealed the anti−oxidative potentials of red palm oil, rooibos and their combination in diabetic conditions and hence, they could be useful in the management of diabetes and its complications.

1. Introduction

Streptozotocin (STZ) is known for its selective cytotoxicity on pancreatic islet β−cells and has been broadly used to induce diabetes mellitus in experimental rat models[1]. Its diabetogenic action of STZ has been explained to cause the alkylation of DNA, production of nitric oxide and free radicals which leads to decreased insulin biosynthesis[2]. The failure of insulin action or insulin production resulting in hyperglycaemia leads to a number of diabetic complications[3,4]. Diabetes does not only lead to hyperglycaemia but also causes hyperlipidaemia, hyperinsulinemia, hypertension, and atherosclerosis[2].

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Oxidative stress can occur as a result of excess ROS production to the available antioxidant buffering capacity[5]. Elevated levels of glucose can induce oxidative stress through various mechanisms which include glycation, PKC activation and sorbitol pathway[6]. An increase in oxidative glucose metabolism leads to increased mitochondrial generation of the superoxide anion which is converted to hydroxyl radicals and hydrogen peroxide[7,8]. Increased production of reactive oxygen species such as superoxide anion and hydrogen peroxide has been linked with cellular injury due to an increase in lipid peroxidation, DNA damage and protein modification or altered gene expression[9]. Oxidative stress acts on signal transduction and affect gene expression through NF−κB, thereby reducing the expression of antioxidant enzymes[6]. An increase in lipid peroxidation and the reduction in antioxidant enzyme activity have been linked with progression of albuminuria in diabetes[10]. The reduction of oxidative stress in diabetic rats may, in itself, offset hyperglycaemia[11].

Red palm oil is obtained from the fleshy orange−

red mesocarp of the fruit of a tropical plant known as oil palm (*Elaeis guineensis*)[12]. It is reported to contain antioxidants vitamins such as vitamin A (carotenes) and vitamin E (tocopherols and tocotrienols)[13,14] and has been reported to prevent oxidative stress in both *in vitro* and *in vivo* systems[15,16]. Red palm oil contains unsaturated and saturated fatty acids in the ratio that is close to one[13,14]. On the other hand, rooibos (*Aspalathus linearis*) is a rich source of polyphenols that is used in making a mild–tasting tea containing no caffeine and low in tannins compared to green or black teas[17]. It contains different bioactive phenolic compounds which include dihydrochalcones, flavonols, flavanones, flavones, and flavanols[18]. Polyphenols are broadly distributed throughout the plant kingdom and represent an abundant antioxidant component of the human diet[17]. Antioxidants are substances that can directly or indirectly offer protection against adverse effects of xenobiotics, drugs, carcinogens, and toxic radical reactions[19,20]. Various antioxidants either scavenge superoxide and free radicals and/or stimulate the detoxification mechanisms within cells, resulting in the prevention of many pathophysiological processes[20]. The antioxidant activities of vitamins, phenolic compounds and foods containing them have been shown in different in vivo systems[21–25]. Epidemiological evidence suggests that antioxidant properties of phenolic compounds may have health benefits[23]. Total antioxidant capacity has been used for the assessment of antioxidant status which would provide useful information for health care[26]. Prevention of oxidative damage is important for health care because oxidative stress is involved in various diseases[26,27]. The purpose of this study was to investigate the potential modulatory effects of red palm oil (RPO) and rooibos tea extract (RTE) as well as their combined effects on the antioxidant status in STZ–induced diabetic male Wistar rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (176–255 g) were bred and used at the Medical Research Council, Primate Unit, Tygerberg, South Africa. The study was conducted after obtaining Ethical Committee Clearance from Cape Peninsula University of Technology (CPUT/HAS–REC 2010/A002). The rats were maintained in a temperature controlled room of 22–25 ℃, humidity of 45%–55%, 15–20 air changes per hour and on a 12 hour light/dark cycle and rats have free access to standard rat chow. The rats were treated by supplementing their diets with 2 mL red palm oil per day[16] and/ or rooibos tea extract (2 g/100 mL) as the only source of drinking[28] for 7 weeks. The fermented rooibos tea was supplied by Rooibos Ltd (Clanwilliam, South Africa) and the red palm oil used

was Carotino palm fruit oil from Malaysia.

2.2. Preparation of extract from rooibos

Aqueous extracts of fermented rooibos was prepared by the addition of freshly boiled tap water to the leaves and stems (2 g/100 mL). The mixture was allowed to stand for 30 min at room temperature, cooled, filtered and dispensed into clean small bottles.

2.3. Induction of diabetes mellitus

Diabetes was induced by a single intramuscular injection of STZ (Sigma–Aldrich, South Africa) at the dose of 50 mg/kg of body weight into overnight fasted rats. Streptozotocin was dissolved in 0.1 M citrate buffer (pH 4.5). Diabetes was confirmed 72 hours after STZ injection by determining the blood glucose levels using an Accu chek glucometer. Only diabetic rats with blood glucose levels above 14 mmol/L were used for the experiment.

2.4. Study design

The rats were divided into five groups consisting of seven rats for the normal control group and eight rats each for the diabetic groups.

Group 1 (Normal control): Rats received a single intramuscular injection of citrate buffer and given tap water orally for 7 weeks.

Group 2 (Diabetic control): Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and given tap water for 7 weeks.

Group 3: Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and treated with RPO (2 mL/day) for 7 weeks.

Group 4: Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and fed with RTE (2 g/100 mL) for 7 weeks.

Group 5: Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and fed with both RPO (2 mL/day) and RTE (2 g/100 mL) for 7 weeks.

At the end of experimental period, all the rats were fasted overnight and sacrificed. Blood was collected from the dorsal aorta by using a 10 mL syringe and transferred into EDTA tubes for plasma collection. The serum and plasma were separated after centrifugation at 3 000 rpm for 15 min and then transferred into properly labelled vials. Liver tissues were excised, rinsed in saline solution, blotted on filter paper and weighed. All the samples collected were stored at −80 ℃ until analysis was performed. The percentage weight of the liver was calculated with the formula below:

$$\text{The percentage weight of organ} = \frac{\text{Absolute weight of organ}}{\text{Final body weight of}} \times 100$$

2.5. High performance liquid chromatography (HPLC) analysis of aqueous rooibos tea extract

The flavonoids in the rooibos tea extract were separated using HPLC (Agilent Technologies, USA) technique according to the method of Bramati et al[29]. The mobile phase was made up of water (A) containing 300 μL/L trifluoroacetic acid and methanol (B) containing 300 μL/L trifluoroacetic acid. The gradient elution started at 95% (A) changing to 75% (A) after 5 min and to 20% (A) after 25 min and back to 95% (A) after 28 min. The flow rate, the injection volume and the column temperature were set at 0.8 mL/min, 20 μL and 23 ℃ respectively. The wavelengths were set between 210 nm and 400 nm and peaks were identify based on the retention time of the standards and confirmed by comparison of the wavelength scan spectra.

2.6. Conjugated diene determination

Conjugated dienes (CDs) concentrations in the samples were determined as described by Recknagel & Glende[30]. Thawed liver and plasma samples (100 μL) were mixed with 405 μL of a chloroform/ ethanol mixture (2:1) and kept on ice. Solutions were vortexed (1 min) and centrifuged (10 000 g; 10 min; 4 ℃). The bottom organic chloroform layers were dried under nitrogen (N_2) gas for 10 min. To each of the dried residues, cyclohexane (1 mL) was added and vortexed. Thereafter, 300 μL of the solution and cyclohexane as blank were transferred into 96–well microplates and the absorbance was determined at 234 nm spectrophotometrically. The CD calculations were done according to the equation given below and expressed as nmol CD/L for plasma and nmol CD/g tissue for liver homogenates.

$$\frac{A_{234}s - A_{234}b}{\xi} \times 100$$

Where A_{234}s: absorbance of sample at 234 nm
A_{234}b: absorbance of blank at 234 nm
ξ : coefficient of extinction= 2.95×10^4
Quoted ξ is based on a 1 cm cuvette; since 300 μL in a microplate well has a length of 0.9 cm, appropriate factoring was done in the calculations.

2.7. Antioxidant enzymes assay

The activities of antioxidant enzymes in the liver were determined. Liver homogenates (10% w/v) were prepared in a phosphate buffer, centrifuged at 10 000 g (4 ℃) for 10 mins and supernatant kept at −80 ℃ for enzyme analyses. Catalase (CAT) activity was determined spectrophotometrically at 240 nm by monitoring the decomposition of H_2O_2 and expressed as μ mole H_2O_2/min/ μ g protein according to the method of Aebi[31] while superoxide dismutase (SOD) activity was determined by the method of Crosti et al[32] modified for a microplate reader at 490 nm and expressed as the amount of protein (μ g) required to produce a 50% inhibition of auto–oxidation of 6–hydroxydopamine. Glutathione peroxidase (GPx) activity was measured spectrophotometrically (340 nm) by the method of Ellerby and Bredesden[33] and the activity expressed as nmoles NADPH/min/ μ g protein.

2.8. Oxygen radical absorbance capacity (ORAC) assay

The plasma samples were deproteinised using 0.5M perchloric acid (PCA), centrifuged at 15 000 g for 10 min. The ORAC assay was conducted according to the method of Ou et al[34] on a 96–well microplate using a Fluorescence plate reader (Thermo Fisher Scientific, Waltham, Mass., USA). The reaction consisted of 12 μL of diluted sample and 138 μL of fluorescein (14 μM), which was used as a target for free radical attack. The reaction was initiated by the addition of 50 μL AAPH (768 μM) and the fluorescence (emission 538 nm, excitation 485 nm) recorded every 1 min for 2 hours. Trolox was used as the standard and results expressed as μ mol/L.

2.9. Trolox equivalence antioxidant capacity (TEAC) assay

Trolox equivalence antioxidant capacity was determined using the principle of 2, 2′–azino–bis (3–ethylbenzothiazoline–6–sulphonic acid) (ABTS) radical scavenging activity according to a method described by Re et al[35]. ABTS⁺ solution was prepared a day before use by mixing ABTS salt (8 mM) with potassium persulfate (3 mM) and then storing the solution in the dark until the assay could be performed. The ABTS⁺ solution was further diluted with distilled water. Twenty five microlitres (25 μL) of the plasma samples were mixed with 300 μL ABTS⁺ solution in a 96–well clear microplate. The plate was read after 30 min incubation at room temperature in a Multiskan Spektrum plate reader (Thermo Fisher Scientific, USA) at 734 nm. Trolox was used as the standard and results expressed as μ mol TE/L.

2.10. Total glutathione, total protein, albumin and globulin analysis

The levels of total glutathione (GSHt) in the samples were determined according to the method of Asensi et al[36]. The liver samples were homogenized (1:10) in 15% (w/v) trichloroacetic acid (TCA) containing 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 15 000 g for 10 min and the supernatant collected. Total glutathione in the liver homogenates was performed by placing 50 μL of the samples into plate wells

and 50 μL of 5, 5'–dithiobis–2–nitrobenzoic acid (DTNB) was added, followed by 50 μL of glutathione reductase. The reaction was initiated by the addition of 50 μL of nicotinamide adenine dinucleotide phosphate (NADPH) to a final volume of 200 μL. The change in absorbance was monitored at 412 nm for 5 min and levels of GSHt calculated using pure glutathione (GSH) as a standard and expressed as μmole/g tissue for liver samples. Total protein and albumin levels in the serum were measured with kits using an automated chemistry analyzer (Easy RA Medical, USA) according to manufacturer`s instructions. Globulin level was determined by using the formula (Globulin = Total protein − Albumin).

2.11. Estimation of thiobarbituric acid reacting substances (TBARS)

Malondialdehyde (MDA) which is a part of TBARS is commonly used as an indicator of lipid peroxidation[37]. Thiobarbituric acid reacting substances was performed according to a method of Khoschsorur *et al*[38] and modified using a micro plate reader. Fifty microlitres (50 μL) of plasma or liver homogenates was mixed with 375 μL of 0.44 M H_3PO_4 and 125 μL of 42 mM aqueous 2–Thiobarbituric acid and 225 μL of distilled water were added. The mixture was heated in boiling–water in a water bath for 60 mins. After cooling on ice, alkaline methanol (5 mL + 45 mL 1M NaOH) was added to the reaction mixture in ratio (1:1). The samples were centrifuged for 3 mins and absorbance read at 535 nm using a micro plate reader. Malondialdehyde was used as the standard and results expressed as nmol MDA/L for plasma and nmol MDA/g tissue for liver homogenates.

2.12. Statistical analysis

Data were expressed as the means±standard deviations. Significant differences between mean values of different groups were determined by one–way analysis of variance (ANOVA) with MedCalc software. Data not normally distributed was log transformed and analyzed using the Kruskal–Wallis one–way ANOVA on ranks hypotheses. Differences were considered significant at $P<0.05$.

3. Results

3.1. Polyphenolic composition of the rooibos tea extract

The flavonoids present in the rooibos tea extract are shown in Figures 1 and 2. The results showed the presence of flavonoids which include aspalathin, isovitexin, isoorientin, hyperoside/ rutin, luteolin and vitexin.
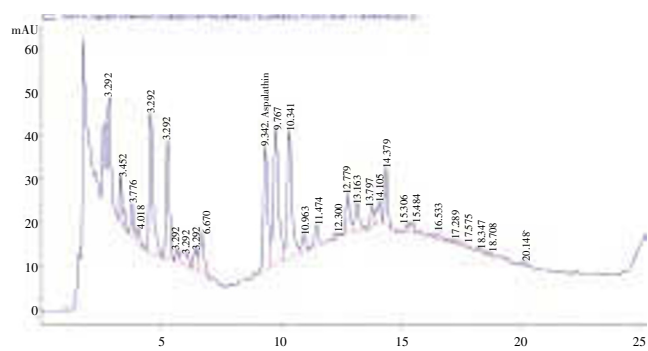
Figure 1. HPLC chromatogram of flavonoid (Aspalathin) in rooibos tea extract used in the study at 287 nm.
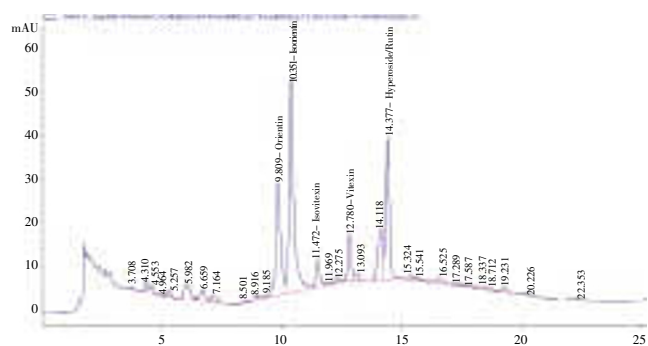
Figure 2. HPLC chromatogram of other flavonoids in rooibos tea extract used in the study at 360 nm.

3.2. Body and liver weight

Effects of RPO and/or RTE treatments on the percentage weight of the liver and body weight gain in diabetic treated rats is shown in Table 1. A significant ($P<0.05$) increase in the liver weights of all the diabetic groups was noted when compared with the normal control group. The results showed a decrease in body weight gain in all the diabetic groups when compared with the normal control group.

Table 1

Effect of RPO, RTE and RPO + RTE treatments on the liver weight and body weight gain.

Treatment groups	Liver weight (%)	Body weight gain(%)
Normal control	3.10±0.53	74.00±11.19
STZ control	4.32±0.41[a]	1.64±12.48 [a]
STZ + RPO	4.59±0.62[b]	17.37±15.66 [b]
STZ + RTE	4.77±0.41[bc]	5.79±19.45 [b]
STZ + RPO + RTE	4.00±0.72[b]	27.44±13.51 [bc]

All significant differences are at $P<0.05$. [a] represents significant difference between STZ control group and normal control group. [b] represents significant difference between RPO or/and RTE treated groups and normal control group. [c] represents significant difference between RPO or/and RTE treated groups and STZ control group.

3.3. Plasma antioxidant capacity

The effect of RPO and/or RTE treatments on the antioxidant capacity in the plasma of the diabetic rats is shown in Table

2. The plasma TEAC status did not show any difference in all the groups in comparison to the normal and STZ control groups. There was a significant ($P<0.05$) decrease in the plasma ORAC status of the STZ control group when compared with the normal control group while it significantly ($P<0.05$) increased in the diabetic rats treated with RPO, RTE and RPO + RTE.

Table 2

Effect of RPO, RTE and RPO + RTE treatments on the plasma antioxidant capacity.

Treatment groups	TEAC(μ mol/L)	ORAC (μ mol/L)
Normal control	6858.83±100.48	613.91±39.58
STZ control	6846.17±293.21	567.83±55.60[a]
STZ + RPO	6799.22±483.27	668.48±50.78[c]
STZ + RTE	7306.43±515.61	664.48±60.74[c]
STZ + RPO + RTE	7081.01±339.59	641.57±88.69[c]

All significant differences are at $P<0.05$. [a] represents significant difference between STZ control group and normal control group. [b] represents significant difference between RPO or/and RTE treated groups and normal control group. [c] represents significant difference between RPO or/and RTE treated groups and STZ control group.

3.4. Liver antioxidant enzymes and total glutathione

The effect of RPO and/or RTE treatments on the antioxidant enzymes and total glutathione in the liver of rats is shown in Table 3. Diabetic rats fed with RTE and combined treatment (RPO + RTE) did not indicate any significant effects on liver CAT activity in comparison to the normal control and STZ control groups. Liver GPX activity significantly ($P<0.05$) increased in RPO, RTE and RPO + RTE treated diabetic rats in comparison to the normal control group. There

was a significant ($P<0.05$) increase in liver GPX activity in the diabetic rats treated with RTE and RPO + RTE when compared with the STZ control group. The activity of liver SOD reduced significantly in the STZ control group when compared with the normal control group. A significant ($P<0.05$) increase in the activity of liver SOD was observed in the diabetic rats treated with RPO, RTE and RPO + RTE. No significant ($P>0.05$) decrease in the liver GSHt level in all the diabetic groups in comparison to the normal control group was shown.

3.5. Oxidative stress biomarkers

The effect of RPO and/or RTE treatments on the oxidative stress biomarkers in the diabetic rats is shown in Table 4. There were no significant ($P>0.05$) differences in the plasma conjugated dienes in all the groups. Diabetic rats treated with RTE showed a significant ($P<0.05$) reduction in liver CDs in comparison to the normal and STZ control groups. Plasma TBARS significantly ($P<0.05$) increased in the STZ control group and RPO treated diabetic rats while RTE and RPO + RTE did not significantly reduce ($P>0.05$) the level of plasma TBARS when compared with the STZ control group. The level of liver TBARS was not significantly ($P>0.05$) different in all treated diabetic rats when compared with both normal and STZ control groups.

3.6. Total protein, albumin and globulin analysis

The effect of RPO and/or RTE on serum total protein, albumin and globulin is shown in Table 5. No significant ($P>0.05$) decrease in the level of total protein was observed

Table 3

Effect of RPO, RTE and RPO + RTE treatments on the antioxidant enzymes and total glutathione in the liver.

Treatment groups	CAT (μ mol H$_2$O$_2$/min/ μ g protein)	GPx (nmol NADPH/min/ μ g protein)	SOD (units/ μ g protein)	GSHt (μ mol/g tissue)
Normal control	0.621±0.127	0.003±0.000	0.226±0.062	3.56±0.85
STZ control	0.670±0.073	0.003±0.000	0.160±0.028[a]	2.36±1.36
STZ + RPO	0.503±0.038b	0.004±0.000[b]	0.246±0.068[c]	3.39±1.53
STZ + RTE	0.655±0.079	0.004±0.001[bc]	0.293±0.043[c]	2.48±1.45
STZ + RPO + RTE	0.651±0.107	0.005±0.001[bc]	0.339±0.058[bc]	2.87±0.57

All significant differences are at $P<0.05$. [a] represents significant difference between STZ control group and normal control group. [b] represents significant difference between RPO or/and RTE treated groups and normal control group. [c] represents significant difference between RPO or/and RTE treated groups and STZ control group.

Table 4

Effect of RPO, RTE and RPO + RTE treatments on the oxidative stress biomarkers.

Treatment groups	CD		MDA	
	Plasma (nmol/L)	Liver (nmol/g)	Plasma (nmol)	Liver (nmol/g)
Normal control	0.12±0.01	1.21±0.02	12.44±2.51	0.25±0.24
STZ control	0.13±0.01	1.20±0.06	18.70±4.23[a]	0.24±0.02
STZ + RPO	0.13±0.02	1.19±0.03	17.88±3.41[b]	0.25±0.02
STZ + RTE	0.13±0.01	1.14±0.04[bc]	16.34±3.55	0.22±0.03
STZ + RPO + RTE	0.14±0.01	1.19±0.03	15.92±2.78	0.26±0.02

All significant differences are at $P<0.05$. [a] represents significant difference between STZ control group and normal control group. [b] represents significant difference between RPO or/and RTE treated groups and normal control group. [c] represents significant difference between RPO or/and RTE treated groups and STZ control group.

in the STZ control group when compared with the normal control group. There was also a significant ($P<0.05$) increase in the total protein of the diabetic rats treated with RPO alone when compared with the STZ control group. There was significant ($P<0.05$) reduction in the levels of albumin in the STZ control group and diabetic treated groups with RPO and RTE alone when compared with the normal control group. The effect of RTE on the level of albumin in STZ treated rats was significantly ($P<0.05$) lower when compared with the normal and STZ control groups. The results also showed a significant ($P<0.05$) increase in serum globulin in diabetic rats fed with RPO. However, diabetic rats fed with RTE and RPO + RTE did not have any significant ($P>0.05$) effects on globulin when compared with the STZ control group.

4. Discussion

Our results indicated a decrease in body weights of the diabetic rats in comparison to the normal control rats. The decrease in body weight is as a result of loss of tissue proteins and muscle mass in diabetes[39]. It is known that glycosuria causes a significant loss of calories for every gram of glucose excreted and most likely, this loss results in severe weight loss in spite of increased appetite, particularly when it is coupled with loss of muscle and adipose tissue due to excessive breakdown of protein[40]. From our results, diabetic rats fed with RPO and RTE gained more body weight than those of the STZ group. Streptozotocin generates oxygen radicals *in vivo* and cause oxidative damage to pancreas, liver, kidney, and haemopoietic systems[13,41]. The key organ of oxidative and detoxifying processes, as well as free radical reactions is the liver and thus, oxidative stress biomarkers are elevated in the liver at the early stages of many diseases[42].

The mechanism of antioxidant defence against oxidative stress can be classified into: antioxidant, preventative, repair mechanisms and physical defences[43]. Vitamin A, one of the components present in red palm oil acts directly by an intrinsic free radical scavenging mechanism and also inhibits nitric oxide production through inhibition of iNOS gene transcription in different tissues[44]. It has also been documented that α–tocopherol, a form of vitamin E in red palm oil has the ability to terminate chain reactions of polyunsaturated fatty acid free radicals generated by lipid oxidation[45]. Vitamin E has also been reported to act by up–regulating antioxidant enzymes[44]. Similarly, rooibos is known to contain a profile of polyphenols (flavonoids). Possible mechanisms of flavonoids against oxidative stress is by the direct scavenging of free radicals, inhibition of xanthine oxidase, interfering with inducible nitric–oxide synthase, immobilization and firm adhesion of leukocytes to the endothelial wall and by interaction with various enzyme systems[46].

Increased antioxidant capacity confirms the idea of the presence of functional recovery, at least in part, in the antioxidant defence systems in rats during chronic

diabetes[47]. The trolox equivalent antioxidant capacity (TEAC) assay or 2, 2–azinobis (3–ethylbenzthiazoline–6–sulfonic acid) (ABTS) assay is based on scavenging of the ABTS$^+$ radical cation by the antioxidants present in test sample[48]. It evaluates the relative ability of antioxidant to scavenge the ABTS$^+$ generated in aqueous and organic solvent systems[49]. There was no significant difference in plasma TEAC in treated diabetic rats. The ORAC assay is found to give a good index of the total antioxidant capacity in patients with diabetes[50]. A decrease in blood ORAC values are strongly linked with poor glycaemic control in diabetic patients[50–52]. In this study, we observed a similar significant decrease in plasma ORAC status in the STZ group. However, plasma ORAC status of the diabetic rats treated with RPO, RTE and combined treatment (RPO + RTE) was significantly increased and therefore, suggest their ability to boost antioxidant levels in diabetic conditions.

CAT is regarded as a major determinant of hepatic antioxidant status and catalyzes the reduction of hydrogen peroxides and protects the tissue from highly reactive hydroxyl radicals[53]. In this study, no significant difference in the activity of liver CAT was observed in all the diabetic groups. SOD catalyses the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen[54]. The location of SOD in the mitochondria and its position in the antioxidant chain make the enzyme to be particularly important as a slight decrease in SOD is sufficient to provoke cell damage[55]. A significant decrease in the activity of liver SOD in the diabetic rats was observed compared with the normal control group in this study and this could be due to an excessive formation of superoxide anions in the diabetic rats. The decrease in liver SOD could as well be related to inactivation by H_2O_2 or by glycation of enzymes. Kumawat *et al*[56] reported that auto–oxidation of glucose results in the formation of H_2O_2 that inactivates SOD. The results from this study also showed that RPO, RTE and RPO + RTE were able to significantly increase the activity of SOD in the diabetic rats. GPx helps to protect the cell from damage due to free radicals like hydrogen and lipid peroxides and its actions take place in the presence of glutathione, the master antioxidant. GPx metabolizes hydrogen peroxide to water with the usage of reduced glutathione as a hydrogen donor[57]. The activity of liver GPx significantly increased in all diabetic treated rats when compared to the normal control group.

The reaction of hydroxyl radicals and singlet oxygen with the methylene groups of polyunsaturated fatty acids (PUFA) produces conjugated dienes, lipid peroxy radicals and hydroperoxides[58,59]. In this study, there was no significant effect on conjugated dienes in the diabetic control and diabetic treated rats. TBARS was used as a measure of the estimation of MDA in this study. The cytotoxic effects of oxygen free radicals is exerted on membrane phospholipids and lead to the formation of MDA, a product of lipid peroxidation[60] and the levels of MDA reveal the degree of oxidation in the body. Lipid peroxidation could cause protein damage and the inactivation of membrane bound enzymes

either through direct attack by free radicals or through chemical modification by its end products, malondialdehyde and 4–hydroxynonenal[61]. There was a significant increase in the plasma TBARS in STZ diabetic rats while liver. TBARS did not show any difference in the diabetic control group in comparison to the normal control group. A similar result of non–accumulation of TBARS in liver tissue of diabetic rats has been shown[62]. A possible reason for this might be as a result of reduction in lipid content (lipolysis) in cell membranes during the long–term diabetes in the rats[62,63]. Lapshina *et al*[63] further argued that TBARS accumulation, which shows the degree of oxidative stress and antioxidative defense, may be tissue–specific and also depends upon duration of the diabetes conditions. Administration of RTE and RPO + RTE to the diabetic rats reduced the plasma TBARS to a level that is not significantly different from the normal control group.

Indirectly, hyperglycaemia is the cause of GSH depletion and these results in oxidative stress[64]. A decrease in GSH levels could signify an increased utilization due to oxidative stress and elevated activity of GSH protection of cellular proteins against oxidation through the glutathione redox cycle, that could also directly detoxify reactive oxygen species the generated from exposure to STZ[65]. Several studies have reported a decrease in GSH level as an indicator of oxidative stress in diabetic conditions[64,66,67]. However, this study showed no significant reduction in the GSHt levels in the diabetic control and diabetic treated rats compared to normal control group. Singh *et al*[68] reported no significant change in GSH levels either in blood or liver of diabetic animals and in treated diabetic animals, GSH levels were marginally high in both blood as well as the liver. The results indicate no significant increase in the plasma GSH levels in diabetic treated animals. In another study, Sudnikovich *et al*[62] did not observe any appreciable change in GSH levels in diabetic red blood cells or liver tissue when compared to normal rats.

Diabetes mellitus is grossly reflected by intense changes in the protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs[69,70]. Reduction in serum albumin, alpha and beta globulin, plasma albumin/globulin ratio and a concomitant elevation in gamma globulin have been shown in diabetic rats[71]. A reduction in protein content in the serum of diabetic patients has been reported and this is indicated by an increase in the lipid peroxidation and a decreased antioxidant defense system[72]. In diabetes, increased blood nitrogenous substances may be accounted for by the enhanced breakdown of both liver and plasma proteins[69]. The results indicate a significant decrease in the level of albumin while there was no significant decrease in the total protein and globulin levels in the diabetic control group in comparison to the normal control group. The decrease in the albumin level of the diabetic rats could be due to an increased protein glycation. The present study revealed that diabetic rats treated with RPO only improved the level of albumin in the diabetic rats. Similarly, RPO significantly increased the level of globulin

in comparison to both the normal and diabetic control groups. Albumin could exert its antioxidant activity due to its capacity to bind homocysteine, a sulphur–containing amino acid, which results from the catabolism of methionine residues[73].

In conclusion, the study confirms the involvement of oxidative stress in diabetes. The results confirms the ability of the RPO, RTE or the combined treatment (RPO + RTE) to up–regulate the activities of some antioxidant enzymes intracellular activities in diabetic conditions. It also suggests that these plant products could offer protective roles against oxidative damage. The antioxidant beneficial effects of red palm and rooibos could be as a result of inhibition of specific pathways that are activated as a consequence of increased oxidative stress in the progression of diabetes. Therefore, antioxidant therapy in diabetes may therefore be helpful in relieving many symptoms and complications observed in diabetes patients.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

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