Impact of Co-administration of Red Palm Oil (*Elaeis guineensis* Arecaceae) and Rooibos (*Aspalathus linearis* Fabaceae) on Glycaemic Parameters, Liver Function and Key Glycolytic Enzymes in Diabetic Rats

Ademola O Ayeleso¹*, Oluwafemi O Oguntibeju¹ and Nicole L Brooks²

¹Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, ²Department of Wellness Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Cape Town, South Africa

*For correspondence: Email: ademola.ayeleso@gmail.com; Tel: +27840171248

Received: 26 December 2014 Revised accepted: 27 July 2015

Abstract

**Purpose:** To investigate the anti-diabetic effects of red palm oil (RPO), rooibos tea extract (RTE) and their combination (RPO + RTE).

**Methods:** Diabetes was induced by a single administration of streptozotocin (50 mg/kg) and the rats were treated for 49 days. The effects of these plant products on plasma glucose, serum insulin, serum fructosamine, glycosylated haemoglobin (HbIAC), liver enzymes in serum and liver glycolytic enzymes were studied using standard techniques.

**Results:** The combined treatment of RPO and RTE significantly (p < 0.05) decreased the glucose (20.98 ± 6.46 mmol/L to 15.60 ± 5.94 mmol/L), HbIAC (16.74 ± 2.73 % to 12.41 ± 2.25 %), fructosamine (98.61 ± 23.35 mmol/L to 62.52 ± 28.41 mmol/L) levels and increased insulin (0.30 ± 0.09 ng/mL to 0.72 ± 0.21 ng/mL) levels in the diabetic rats. Similarly, the combined treatment significantly (p < 0.05) reduced alanine aminotransferase (ALT) in the serum of diabetic rats. RPO + RTE significantly (p < 0.05) increased the activity of pyruvate kinase in the liver when compared with the diabetic control group.

**Conclusion:** Combined treatment with red palm oil and rooibos shows promising beneficial effects in diabetic conditions of rats. Further studies on the mechanism of actions of the plant products are required.

**Keywords:** Red palm oil, Rooibos, Glycaemic parameters, Liver function, Glycolytic enzymes, Diabetic rats

INTRODUCTION

Diabetes mellitus is a complex disorder arising from various causes which include dysregulated glucose sensing or insulin secretion, autoimmune-mediated β-cell destruction in type 1 diabetes or insufficient compensation for peripheral insulin resistance in type 2 diabetes [1]. Hyperglycaemia (excessive glucose level) is a link between diabetes and diabetic complications [2] and also plays a vital role in increased protein glycosylation [3,4]. Elevated liver enzymes in serum are widespread in diabetic patients and this reflects the severity of hepatic injury [5].
Red palm oil is used as cooking oil in West and Central Africa and it is a source of energy and essential fatty acids in many regions of the world [6]. Red palm oil contains at least 500 ppm carotenoids of which the majority is in the form of α- and β-carotene and approximately 500 ppm vitamin E of which the majority is in the form of tocotrienols [7]. Rooibos (Aspalathus linearis), a South African herbal tea, is made from the leaves and stems of the fynbos plant. Secondary metabolites present in fermented rooibos plant include single ring phenolic acids and monomeric flavonoids such as dihydrochalcones, flavanones, flavones, and flavonols [8-10].

Red palm oil and rooibos are both natural plant products known to have various health promoting benefits which can largely be attributed to their antioxidant properties. The present study was designed to investigate the potential anti-diabetic effects of red palm oil, rooibos and their combined treatment in STZ-induced diabetic rats.

EXPERIMENTAL

Plant products

Fermented rooibos was supplied by Rooibos Ltd (Clanwilliam, South Africa) and the red palm oil used was Carotino palm fruit oil from Malaysia. Aqueous extracts of fermented rooibos tea 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 bottles.

Animals

Male Wistar rats (176 - 255 g) were bred and used at the Medical Research Council (MRC), 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). All the animals received humane care in accordance to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science (NAS) and published by the National Institute of Health (Publication no. 80 – 23, revised 1978). The rats were maintained in a temperature controlled room of 22 - 25 °C, humidity of 45-55 %, 12 h light/dark cycle and have free access to standard rat chow. The rats were treated by supplementing their diets with 2 ml red palm oil [11] and/ or 2 % rooibos [8] for 49 days.

Induction of diabetes mellitus

Male Wistar rats (176 - 255 g) were injected intramuscularly with freshly prepared STZ (50 mg/kg body weight (Sigma Aldrich, South Africa) in 0.1 M citrate buffer (pH 4.5). Blood samples were taken from the tail tip after 72 h of STZ injection and glucose levels determined using an Accu chek glucometer. The rats showing blood glucose levels above 14 mmol/L were selected as diabetic in this experiment.

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 49 days.

Group 2 (diabetic control): Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and the diabetic rats were given tap water for 49 days.

Group 3: Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and the diabetic rats were treated with RPO (2 ml/day) for 49 days.

Group 4: Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and the diabetic rats were treated with RTE (2 g/100 mL) for 49 days.

Group 5: Diabetes was induced by a single intramuscular injection of STZ at a dose of 50 mg/kg body weight and the diabetic rats were treated with both RPO (2 ml/day) and RTE (2 g/100 mL) for 49 days.

At the end of experimental period, overnight-fasted rats were sacrificed and blood was collected from the dorsal aorta using a syringe into an EDTA tube for whole blood, sodium fluoride/potassium oxalate tube for plasma (glucose determination) and serum separator tube for serum collection. The serum and plasma were separated after centrifugation at 3,000 rpm for 15 min and then transferred into properly labelled vials and stored at -80 °C until the analysis was carried out. The liver tissues were excised, rinsed in saline solution, blotted on filter paper, weighed and stored in the freezer at -80 °C.
Glucose determination

The level of glucose was evaluated with a kit using a clinical chemistry analyzer (EasyRA Medical, USA) according manufacturer’s instructions.

Determination of insulin, glycosylated haemoglobin and fructosamine

Serum insulin level was determined with a rat insulin radioimmunoassay kit (Millipore, USA). The glycosylated haemoglobin (HbA1c) in whole blood and serum fructosamine levels were determined with kits (Diazyme Laboratories, USA) using a chemistry analyser (Vitalab Selectra E) according to manufacturer’s instructions.

Liver function assay

The liver function enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) were evaluated in the serum with kits using a clinical chemistry analyzer (EasyRa medical, USA) according manufacturer’s instructions.

Glycolytic enzymes assay

Pyruvate kinase (PK) and glucokinase activity (GK) activities in the liver were determined with colorimetric assay kits (Bio Vision, USA) and enzyme immunoassay technique kits (Cusabio, China) respectively.

Statistical analysis

Data are expressed as means (in duplicates) ± standard deviation (SD). Significant differences between mean values of different groups were determined by one-way analysis of variance (ANOVA) using MedCalc v 12.2.1 software (MedCalc software bvba, Belgium). Data not normally distributed was log transformed and analyzed using Kruskal - Wallis one-way ANOVA on ranks hypotheses. Differences were considered significant at $p < 0.05$.

RESULTS

Glucose, insulin, Hb1Ac and fructosamine

There was a significant ($p < 0.05$) increase in the level of Hb1Ac in all the diabetic rats when compared with the normal control group (Fig 3). Combined treatment (RPO + RTE) significantly ($p < 0.05$) reduced the Hb1Ac level when compared with the STZ control group while diabetic rats treated with either RPO or RTE alone did not show any significant ($p > 0.05$) difference in the levels of Hb1Ac when compared with the STZ control group. Fructosamine was significantly ($p < 0.05$) increased in all the diabetic groups when compared with the normal control group. However, treatment with RPO + RTE significantly ($p < 0.05$) reduced fructosamine in comparison to the STZ control group.

Liver function

Serum ALT, AST and ALP were significantly ($p < 0.05$) increased in all the diabetic groups in comparison to the normal control group. There was a significant ($p < 0.05$) decrease in ALT in diabetic rats treated with RPO + RTE when compared with the STZ control group. There was no significant ($p > 0.05$) effect on ALP in diabetic rats treated with RPO, RTE and RPO + RTE when compared with the STZ control group. GGT was not detectable in the serum of all normal control and normal treated groups. Similarly, diabetic rats treated with RPO and RPO + RTE did not show the presence of GGT while it was found in the serum of STZ control group as well as the diabetic rats treated with RTE only.

Glycolytic enzymes

The activity of pyruvate kinase was significantly ($p < 0.05$) reduced in the STZ control group as well as treated diabetic groups in comparison to the normal control group (Table 1). Diabetic rats treated with RPO and RTE alone did not show significant ($p > 0.05$) increase in pyruvate kinase activity. The activity of pyruvate kinase in the diabetic rats was significantly increased by RPO + RTE supplementation when compared with STZ control group. There was no significant ($p > 0.05$) difference in the activity of glucokinase in all the groups (Table 1).
**Fig 1:** Effect of RPO and/or RTE treatments on blood glucose level. All significant differences are at $p < 0.05$. STZ, streptozotocin-induced diabetes; RPO, red palm oil; RTE, rooibos tea extract; $^a$ significant difference between STZ control group and normal control group; $^b$ significant difference between RPO and/or RTE treated groups and normal control group; $^c$ significant difference between RPO and/or RTE treated groups and STZ control group.

**Fig 2:** Effect of RPO and/or RTE treatments on insulin level. All significant differences are at $p < 0.05$. STZ, streptozotocin-induced diabetes; RPO, red palm oil; RTE, rooibos tea extract; $^a$ significant difference between STZ control group and normal control group; $^b$ significant difference between RPO and/or RTE treated groups and normal control group; $^c$ significant difference between RPO and/or RTE treated groups and STZ control group.

**Fig 3:** Effect of RPO and/or RTE treatments on the level of Hb1Ac. All significant differences are at $p < 0.05$. STZ, streptozotocin-induced diabetes; RPO, red palm oil; RTE, rooibos tea extract. $^a$ significant difference between STZ control group and normal control group; $^b$ significant difference between RPO and/or RTE treated groups and normal control group; $^c$ significant difference between RPO and/or RTE treated groups and STZ control group.
Fig 4: The effects of RPO and/or RTE treatments on the level of fructosamine. All significant differences are at $p < 0.05$. STZ, streptozotocin-induced diabetes; RPO, red palm oil; RTE, rooibos tea extract; $^a$ significant difference between STZ control group and normal control group; $^b$ significant difference between RPO and/or RTE treated groups and normal control group; $^c$ significant difference between RPO and/or RTE treated groups and STZ control group.

Table 1: Effects of RPO, RTE and RPO + RTE treatments on liver function and glycolytic enzymes

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>AST U/L ±</th>
<th>ALT U/L ±</th>
<th>ALP U/L</th>
<th>GGT U/L</th>
<th>Pyruvate kinase mU/mg tissue</th>
<th>Glucokinase ng/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROL</td>
<td>64.96 ± 9.38</td>
<td>45.60 ± 8.41</td>
<td>72.00 ± 15.52</td>
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<td>32.18 ± 2.29</td>
<td>3.67 ± 0.09</td>
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<tr>
<td>STZ CONTROL</td>
<td>166.96 ± 129.75</td>
<td>110.58 ± 62.90</td>
<td>205.18 ± 112.09</td>
<td>6.40 ± 1.67</td>
<td>16.24 ± 4.07</td>
<td>3.61 ± 0.11</td>
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<td></td>
<td>$^a$</td>
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</tr>
<tr>
<td>STZ + RPO</td>
<td>121.17 ± 60.59</td>
<td>102.38 ± 72.28</td>
<td>224.31 ± 70.65</td>
<td>-</td>
<td>17.44 ± 1.87</td>
<td>3.66 ± 0.14</td>
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<td></td>
<td>$^b$</td>
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<tr>
<td>STZ + RTE</td>
<td>131.84 ± 69.81</td>
<td>86.81 ± 62.9</td>
<td>246.75 ± 70.65</td>
<td>6.80 ± 1.87</td>
<td>17.0 ± 5.22</td>
<td>3.57 ± 0.10</td>
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<tr>
<td>STZ + RPO + RTE</td>
<td>84.93 ± 49.45</td>
<td>64.88 ± 33.01</td>
<td>216.88 ± 132.53</td>
<td>-</td>
<td>26.48 ± 5.87</td>
<td>3.62 ± 0.06</td>
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<tr>
<td></td>
<td>$^c$</td>
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All significant differences are at $p < 0.05$. STZ, streptozotocin-induced diabetes; RPO, red palm oil; RTE, rooibos tea extract; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transpeptidase. $^a$ significant difference between STZ control group and normal control group. $^b$ significant difference between RPO and/or RTE treated groups and normal control group. $^c$ represents significant difference between RPO and/or RTE treated groups and STZ control group.

DISCUSSION

It has been suggested that experimental animal models are one of the best ways to understand the pathophysiology of any disease [12-14]. In this study, the intra-muscular administration of streptozotocin effectively induced diabetes mellitus in rats which was confirmed by elevated levels of blood glucose. The biological effects of STZ may be ascribed to its hydrophilicity, structural similarity with glucose and alkylation [14]. Administration of RPO and RTE alone to the animals did not prevent loss of body weight in STZ-diabetic rats. However, the combined treatment with RPO + RTE was able to increase the body weight when compared with the STZ control group. From our results, the increased levels of plasma glucose in the diabetic rats treated with RPO and RTE alone were not significantly different from the diabetic control group.

However, there was a significant reduction in glucose level in the diabetic rats with the combined treatment (RPO + RTE). Similarly, RPO + RTE significantly increased insulin level in the diabetic rats in comparison to the diabetic group. Our results reveal the anti-hyperglycaemic
The liver plays a vital role in carbohydrate metabolism regulation and liver function tests are frequently used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs [5]. Serum aminotransferases such as ALT indicates the concentration of intracellular hepatic enzymes that have seeped out into circulation which serves as a marker of hepatocyte injury [18]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities are used as the indicators of hepatocytes damage [19,20]. Primarily, ALT is mainly found in the liver but AST can be found in the liver and some other organs, so it is a less-specific marker for liver toxicity [19,20]. Our results showed elevated levels of AST, ALT, ALP, and GGT in the serum of the diabetic rats.

The combined treatment (RPO + RTE) was able to reduce the level of ALT significantly in the diabetic rats. This reduction in ALT reveals the potential benefits of combined RPO + RTE treatment in the prevention of liver injury and this reduction could be as a result of the antioxidant properties of the combined RPO and RTE. The gamma-glutamyl transeptidase (GGT), another liver enzyme acts as a marker of biliary function and cholestasis [18]. From our results, GGT was found in the serum of the diabetic rats and the fact that it could not be detected in the diabetic rats treated with RPO suggests that RPO was able to prevent liver damage by the non-leakage of GGT into the serum.

Based on the results, diabetic rats treated with either RPO or RTE did not show any significant difference while those treated with the combination of RPO + RTE showed a significant increase in the activity of pyruvate kinase in the STZ diabetic rats when compared with the STZ control group. Glucokinase is an enzyme catalyzing the phosphorylation of glucose and other hexoses by means of phosphoryl donors (ATP, ADP, and inorganic polyphosphate) and related homologously and by evolution to at least three other hexokinases [22]. Glucokinase in the liver is an essential regulator of glucose storage and disposal [23,24] and its activity was decreased in the liver of diabetic rats which may be due to a deficiency of insulin [24]. In this study, glucokinase was not altered in the STZ induced diabetic rats. It has been previously reported that glucokinase, which plays a role in the generation of a metabolic signal for glucose induced secretion of insulin, is not actively involved in mediating STZ toxicity [25].

CONCLUSION

The results indicate that continuous administration of antioxidant compounds of plant origin play a complementary role in the management of metabolic diseases such as diabetes mellitus. The combined treatments of red palm oil and rooibos was able to show more significant beneficial effects in the management of diabetes and this may be due to the combination of different antioxidants (both fat soluble and water soluble) that are present in the two plant products. Further studies are
recommended to establish the mechanism of actions of the plants products.

ACKNOWLEDGEMENT

This work was carried out with funds provided by Cape Peninsula University of Technology, Bellville, South Africa.

REFERENCES