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EFFECTS OF RED PALM OIL AND ROOIBOS ON SPERM MOTILITY PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Background: Diabetes mellitus characterized by hyperglycaemia could affect sperm quality as a result of increased oxidative stress. This study was performed to investigate the effects of red palm oil (RPO), aqueous rooibos tea extracts (RTE) as well as their combination (RPO + RTE) on sperm motility parameters in streptozotocin-induced diabetic rats.
Materials and Methods: Diabetes was induced by a single administration of streptozotocin (50 mg/kg) and the rats were treated with red palm oil (2 ml/day) and / or aqueous rooibos tea extract (2%) for 7 weeks. Sperm motility parameters were measured using Computer Assisted Sperm Analyzer (CASA).
Results: Hyperglycaemia negatively affected the sperm progressive motility significantly at $p<0.05$. There was a significant decrease ($p<0.05$) in sperm linearity (LIN) in the diabetic group when compared with the normal control group. RPO supplemented diabetic rats exhibited increased progressive sperm motility, sperm linearity (LIN) and wobble (WOB). Significant decreases ($p<0.05$) in straight line velocity (VSL) and average path velocity (VAP) of the sperms were observed in all the diabetic groups when compared to the control group. Significant ($p<0.05$) elevated levels of WOB and LIN were observed following RTE treatment and co- administration with RPO respectively.
Conclusion: The present study suggests that red palm oil and / or rooibos administration exhibited no adverse effects on sperm motility parameters but rather showed some beneficial effects.

Key words: Red palm oil, Rooibos, Sperm, Rats, Streptozotocin, Diabetes mellitus

Introduction

Diabetes Mellitus (DM) is a state of chronic hyperglycaemia and a major cause of micro and macrovascular diseases which affects nearly every system in the body (Amaral *et al*., 2008). Oxidative stress is a usual consequence of hyperglycaemia (Amaral *et al.,* 2008; Fernandes *et al*., 2011) and it is one of the most important factors that contribute to poor semen quality (Bucak *et al*., 2010; Bansal and Bilaspuri, 2010). Oxidative stress is an imbalance between the production of reactive oxygen species and a biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage (Agarwal *et al*., 2003; Bansal and Bilaspuri, 2010). The functional capability of sperm and the production of an adequate number of sperm is an absolute requirement from the male to ensure fertilization (Lifeng *et al*., 2006). Diabetes is linked with decline in sexual function in both male and female persons (Mallick *et al*., 2007; Kim and Moley, 2008). Diabetes may affect male reproductive function at multiple levels due to its effects on the endocrine control of spermatogenesis itself or by impairing penile erection and ejaculation (Sexton and Jarow, 1997; Agbaje *et al*., 2007).

At present, according to the World Health Organization, approximately 80% of the world's population depends on indigenous or traditional medicines for their primary health needs (Pantsi *et al*., 2011). Red palm oil (RPO) which originated from the tropical area of Africa is natural oil obtained from the fleshy orange–red mesocarp of the fruit of palm tree (*Elaeis guineensis*). It contains lipid-soluble antioxidants such as carotenoids (α- and β- carotenes, lycopenes), vitamin E (in the form of α-, β-, δ- tocotrienols and tocopherol) and ubiquinone (Oguntibeju *et al*., 2009). Red palm oil is distinctive from other plant and animal oil because it contains 50% saturated fatty acids, 40% unsaturated fatty acids, and 10% polyunsaturated fatty acids (Atawodi *et al*., 2011). RPO has beneficial effects on arterial thrombosis and hypertension associated with oxidative stress (Edem, 2002; Narang *et al*., 2004) and is also protective against the consequences of ischemia /reperfusion injury (Esterhuyse *et al*., 2005, Bester *et al*., 2006). Aboua *et al*. (2009) showed that RPO could possibly inhibit apoptosis in rat sperm while its role in reducing oxidative stress in HIV/AIDS and tuberculosis patients has been reported (Oguntibeju *et al*., 2010).

On the other hand, rooibos, an indigenous South African herbal tea, is made from the leaves and stems of the fynbos plant, *Aspalathus linearis* and its popularity as a health beverage is known both locally and internationally (Marnewick *et al*., 2011). The herbal tea is prepared from both the unfermented "green" and fermented "oxidised" plant material (Beelders *et al*., 2012). Secondary metabolites found in fermented rooibos include single ring phenolic acids and monomeric flavonoids such as dihydrochalcones, flavanones, flavones, and flavonols (Joubert *et al*., 2008; Beelders *et al*., 2012). Rooibos is also rich in flavonoids which include aspalathin, isoorientin, and nothofagin (Kazuno *et al*, 2005). Some other flavonoids such as luteolin, chrysoeriol, quercetin, isoquercetin and hyperoside in rooibos have also been reported (Jobert *et al*., 2008). Some of the health benefits of rooibos include anti-ageing (Inanami et al., 1995), anti–HIV (Nakano *et al*., 1997), anti-mutagenic (Standley *et al*., 2001), hepatoprotective (Ulicna *et al*., 2003), anti-spasmodic (Gilani *et al*., 2006), anti-cancer (Marnewick *et al*., 2005; 2009), anti-inflammatory (Baba *et al*., 2009), cardio protective effects (Pantsi et al., 2011) and anti-oxidative effects on reproductive functions (Awoniyi *et al*., 2011; 2012). Aspalathin, a major flavonoid in rooibos has also shown anti- diabetic potentials (Kawano *et al*., 2009). The present study was designed to investigate the effects of rooibos, red palm oil and their combined treatment on sperm motility parameters in STZ-induced diabetic rats.

Materials and Methods

Preparation of Rooibos tea extract

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 bottles.

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Experimental 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). The rats were maintained in a temperature controlled room of 22-25 °C, 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 (Aboua et al., 2009) and/ or 2% rooibos tea extracts (Marnewick et al., 2009) for 7 weeks. The fermented rooibos was supplied by Rooibos Ltd (Clanwilliam, South Africa) and the red palm oil used was Carotino palm fruit oil from Malaysia.

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. Rats with blood glucose levels above 14 mmol/L were considered diabetic and used for the experiment.

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. (2002). 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 23oC respectively. The wavelengths were set between 210nm and 400 nm and peaks were identified based on the retention time of the standards and confirmed by comparison of the wavelength scan spectra.

Analysis of sperm motility

Sperm motility of each sample was measured by means of CASA using the Sperm Class Analyzer (SCA) (Microptic, Barcelona, Spain). The following sperm motility parameters were determined: curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), linearity (LIN), straightness (STR) and wobble (WOB).

Experimental design

The rats were divided into five (5) groups consisting of six rats (6) for normal control and eight (8) for 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%) 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%) for 7 weeks.

Results and Discussion

Table 1 shows the nutritional composition (per 100 ml) of red palm oil used as adapted from the nutritional label of the Carotino Palm Fruit Oil from Malaysia. It also shows the nutritional composition of the red palm oil consumed daily by the rats.

Table 1: **Nutritional Composition of Carotino Red Palm Oil used in this study.**

	Per 100 ml	2ml RPO / day
Energy	3400 KJ	68 KJ
Total Fat	92 g	1.84 g
Monounsaturates	43 g	0.86 g
Polyunsaturates	12 g	0.24 g
Saturates	37 g	0.74 g
Trans fat	0 g	0 g
Natural Carotenes		0.92 g
Beta Carotene		0.44 mg
Alpha Carotene		0.34 mg
Other Carotenes		0.146 mg
Cholesterol, Sodium	0 mg	0 mg
Protein, Carbohydrate, Dietary Fibre	0 g	0 g
Co- Enzyme Q10	4.0 mg	0.08 mg

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Table 2: HPLC quantification of flavonoids in aqueous rooibos tea extract used in this study.

Flavonoids	Concentration (µg/mL)
Aspalathin	12.51
Isovitexin	3.50
Iso orientin	23.35
Hyperoside/ rutin	17.73
Luteolin	0.12
Vitexin	5.65

Table 3: Linearity (LIN), straightness (STR), Wobble (WOB) of the sperm cells in the rats.

Treatment Groups	Normal Control	Diabetic Control	Diabetes + RPO	Diabetes + RPO	Diabetes + RPO + RTE
LIN (%)	24.68 ± 1.73	18.68 ± 1.50[a]	21.45 ± 1.23	22.50 ± 0.70	23.77 ± 1.31[b]
STR (%)	45.92 ± 1.82	40.15 ± 1.79	43.47 ± 1.58	42.82 ± 0.69	43.30 ± 1.04
WOB (%)	53.37 ± 1.85	49.08 ± 1.36[a]	51.93 ± 1.42	52.27 ± 0.37[b]	51.37 ± 1.12

RPO (red palm oil), RTE (aqueous rooibos tea extract). All significant differences are at $p<0.05$. [a] Values differ significantly from the normal control. [b] Values differ significantly from the diabetic control.

Streptozotocin-induced diabetes in male rats led to atrophy of sex organ, changes in histoarchitectecture of ventral prostate, diminution in sperm count with low levels in plasma gonadotrophins and testosterone (Mallick et al., 2007). Short and long-term streptozotocin -induced diabetic rats has been reported to show significant decrease in sperm count, motility and morphology (Hassan et al**.,** 1993; Amaral et al**.,** 2006; Rama Raju et al., 2012). Elevated oxidative stress is broadly known to participate in the development and progression of diabetes and its complications (Maritim et al., 2003) and decreased motility in diabetic patients is attributed to increased oxidative stress by ROS (Rama *et al*., 2012). The mammalian spermatozoon is chiefly susceptible to oxidative stress due to lack of cytoplasm, composition of the plasma (rich in polyunsaturated fat acids) and exposure to different environments (Mata-Campuzano et al., 2012). Nearly all cellular components such as proteins, polyunsaturated fatty acids, mitochondrial and nuclear DNA are targeted by ROS and causes genetic modifications in mitochondrial DNA such as deletions and point mutations that are associated with impaired sperm motility (Rama Raju et al., 2012).

Computer-aided sperm analysis (CASA) is a sensitive tool in reproductive research which has been widely used in motility analysis of rat and human spermatozoa (Mdhluli and Horst van der, 2002). The link between fertility rate and the motility parameters such as VSL, ALH, BCF, VCL, LIN and STR in *in-vitro* fertilizing capacity of rat spermatozoa has been reported (Moore and Akhondi, 1996; Lifeng et al., 2006). The involvement of ROS in male fertility is due to its capacity to induce detrimental chemical and structural modifications to sperm nuclear DNA, damage to the proteins and lipids in sperm and mitochondrial- membranes (Gharagozloo and Aitken, 2011). Antioxidants play an essential role in the maintenance of the motility and genetic integrity of spermatozoa (Hughes et al., 1998; Mata-Campuzano et al., 2012).

Progressive motility is a sensitive parameter for detecting abnormal sperm motion (Horimoto *et al*., 2000). It is expressed as a percentage of progressively motile sperm that did not include fast, but nonlinear, straight but slow sperm (Horimoto et al., 2000). In this study, the results showed a decrease in the progressive motility in the diabetic control group and diabetic rats treated with RTE and in combination with RPO when compared with the normal control group. However, there was an increase though, not significant in the progressive motility of the diabetic group treated with RPO alone. This ability of RPO bringing the progressive motility to near normal indicates that it can be helpful in improving fertility. The beneficial effects of red palm oil on induced-oxidative damage in male reproduction have been reported in previous studies (Aboua et al., 2009; 2012). Velocity parameters such as VCL, VSL and VAP directly express sperm motion known as swimming speed (Horimoto et al., 2000). Curvilinear velocity (VCL) is the addition of the incremental distances moved in each frame along the sampled path divided by the time taken for spermatozoa to cover the track (Brecchia et al., 2010). Straight/line velocity (VSL) is the straight line distance from beginning to end of a sperm track divided by the time taken and average path velocity (VAP) is the average velocity of sperm movement (Bian et al., 2004).

VCL, among the other sperm movement parameters is the most significant and independent CASA parameter that greatly show a relationship in predicting the rate of fertilization in the general male population (Larsen et al., 2000; Moradi et al., 2013). In this study, VCL showed no significant decrease in the diabetic group and treated diabetic groups with RPO and RPO+RTE in comparison to the normal control group (Figure 3). There was significant decrease in VSL and VAP in the diabetic group when compared with the normal control group (Figure 4, 5). Yeung et al., (1992) showed that increase in VAP, VSL and STR is a display of mature spermatozoa. Treated diabetic rats with RPO, RTE and RPO + RTE did not show any significant improvement on the sperm velocity parameters in the diabetic rats (Figure 4, 5). These results show that hyperglycaemia negatively affected the swimming speed of the sperm and the plant products could not bring them the velocity parameters back to normal.

Linearity (LIN) of the sperm movement is the ratio of projected length to total length of curvilinear trajectory (Mdhluli and Horst van der, 2002). The results from this study also showed a significant decrease in LIN in the diabetic control group when compared with normal control group (Table 3). Treated diabetic rats with RPO and RTE alone did not have any significant effect on LIN when compared with both

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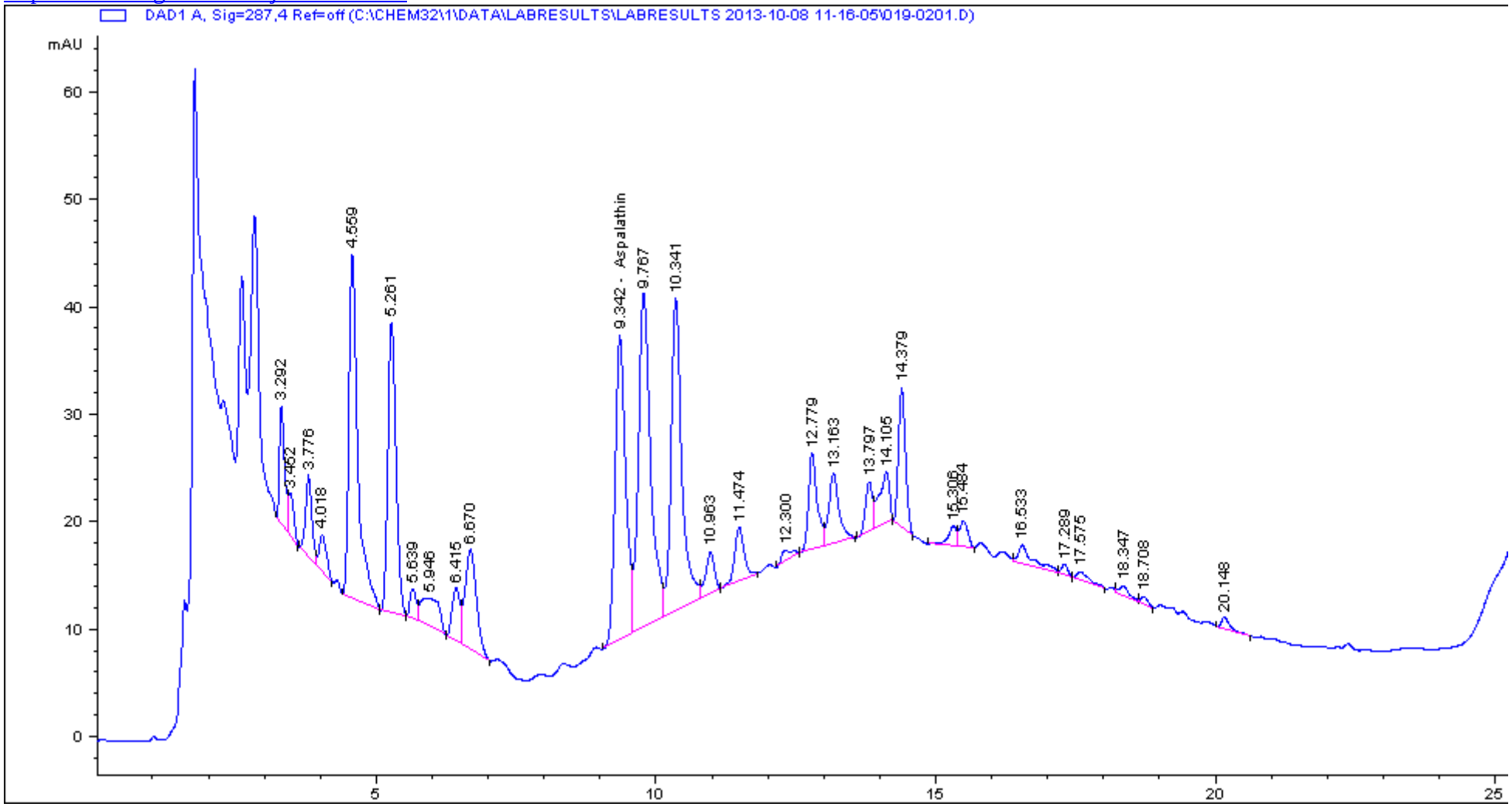

DAD1 A, sig = 287, 4 ref = off

Figure 1a: HPLC chromatogram of flavonoid (Aspalathin) in aqueous rooibos tea extract used in the study at 287 nm.

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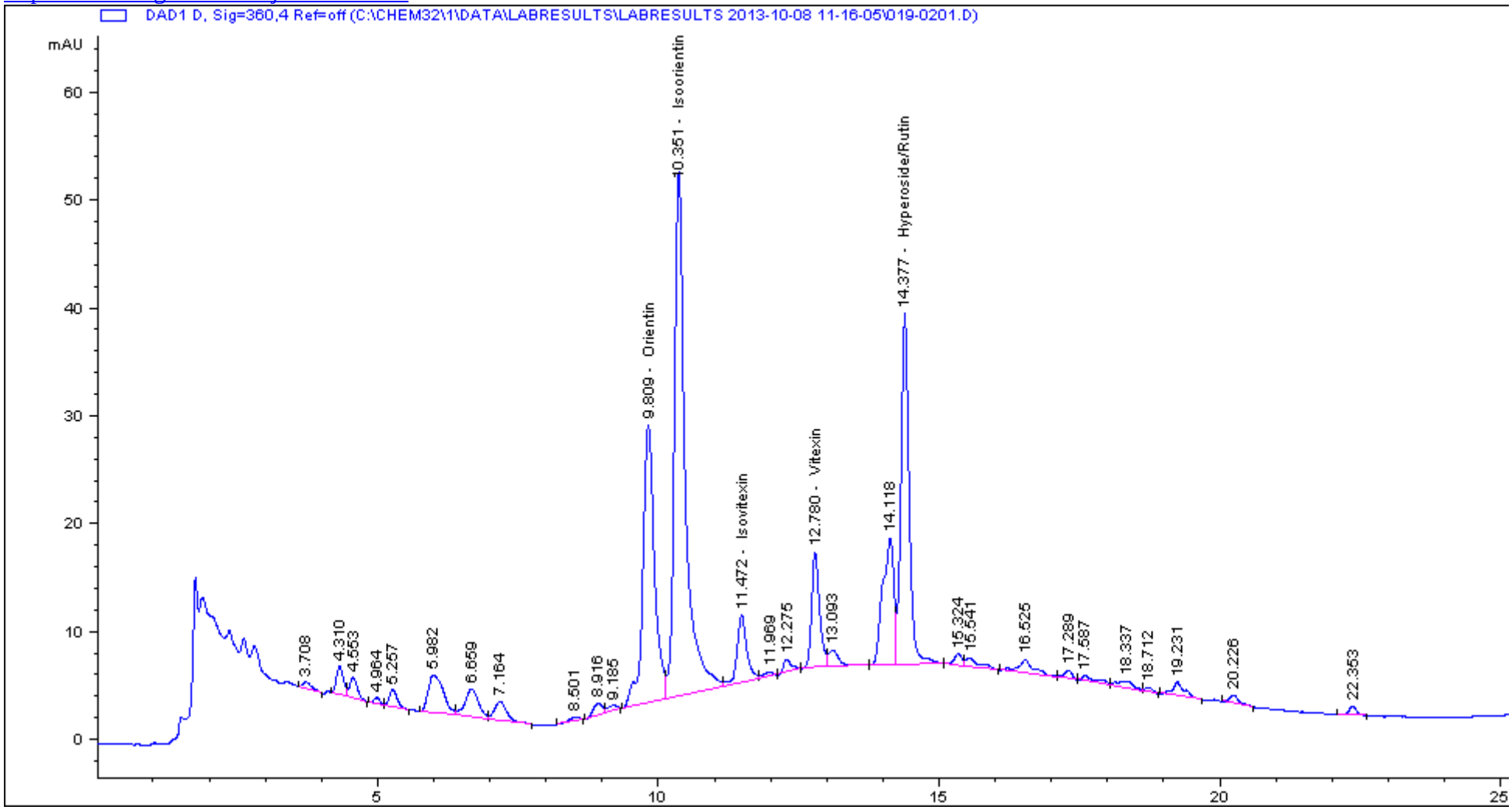

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Figure 1b: HPLC chromatogram of other flavonoids in aqueous rooibos tea extract used in the study at 360 nm.

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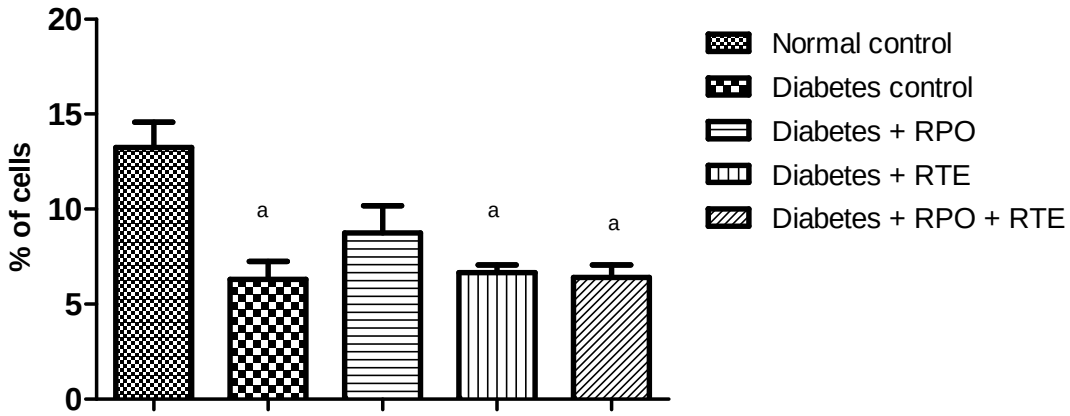

Figure 2: Percentage progress motility of the sperm cells in the rats.
RPO (red palm oil), RTE (aqueous rooibos tea extracts). All significant differences are at $p<0.05$. [a] Values differ significantly from the normal control.

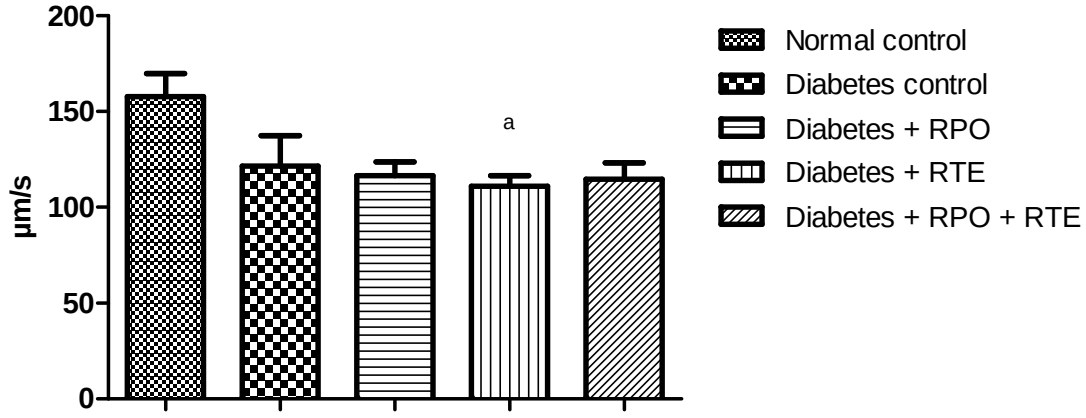

Figure 3: Curvilinear velocity (VCL) of the sperm cells in the rats.
RPO (red palm oil), RTE (aqueous rooibos tea extracts). All significant differences are at $p<0.05$. [a] Values differ significantly from the normal control.

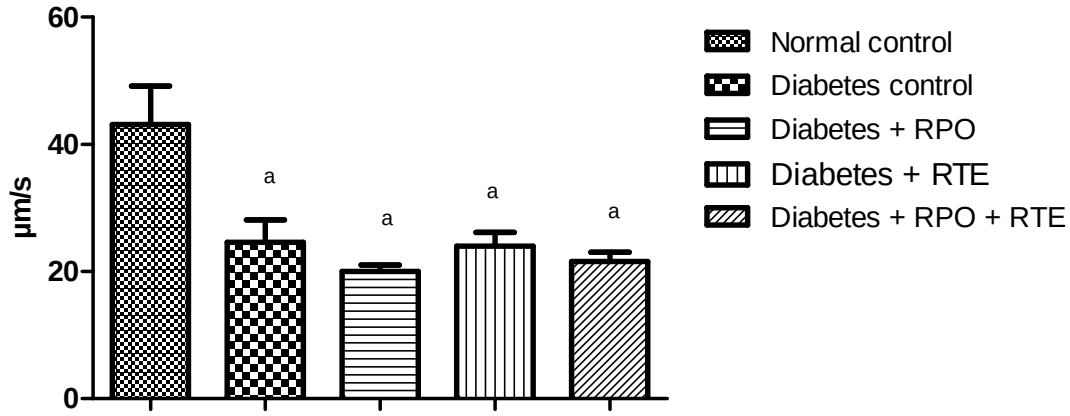

Figure 4: Straight line velocity (VSL) of the sperm cells in the rats.
RPO (red palm oil), RTE (aqueous rooibos tea extracts). All significant differences are at $p<0.05$. [a] Values differ significantly from the normal control.

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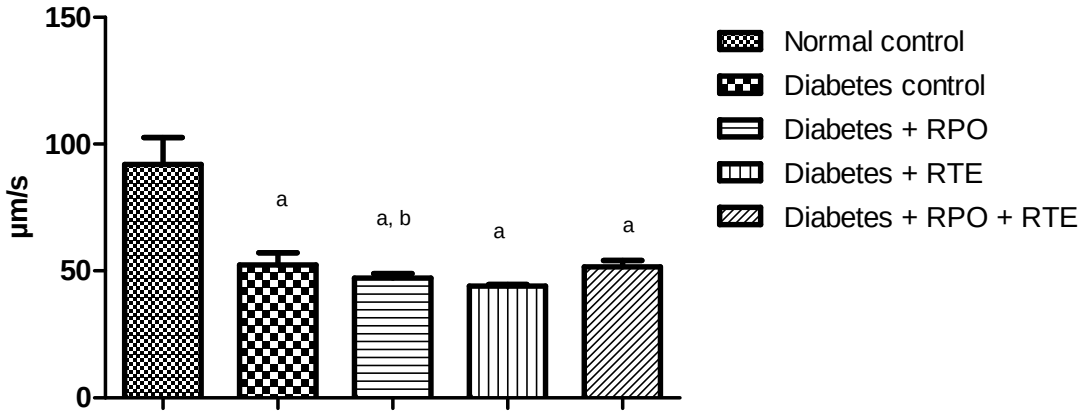

Figure 5: Average path velocity (VAP) of the sperm cells in the rats.
RPO (red palm oil), RTE (aqueous rooibos extract). All significant differences are at $p<0.05$. [a] Values differ significantly from the normal control. [b] Values differ significantly from the diabetic control.

Normal and diabetic control groups (Table 3). However, there was a significant improvement on LIN after RPO + RTE supplementation in comparison to the diabetic control group, indicating the protective effect of the combined treatment of the plants products (Table 3). Wobble (WOB) of sperm movement is the expression of the degree of oscillation of the curvilinear path about its spatial average path (Mdhluli and Horst van der, 2002) and straightness (STR) measures the departure of sperm cell from a straight line (Estienne et al., 2007). There were no significant effects on the STR and WOB in the diabetic control group when compared with normal control group (Table 3). Treatment of diabetic rats with RPO, RTE and RPO+RTE did not show any significant difference on STR in comparison to the normal control and diabetic control groups (Table 3). Diabetic rats treated with RTE significantly increased WOB when compared with the diabetic control group while RPO and RPO + RTE brought WOB to almost normal (Table 3).

In conclusion, prolonged increase in blood glucose significantly altered the characteristics of sperm motility while dietary supplementation with red palm oil and rooibos in the diabetic rats demonstrated an improvement in sperm motility parameters, suggesting its possible beneficial effects on sperm functions.

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