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RESEARCH ARTICLE

MJM BIOLABS

The Effect of Diosmin-Hesperidin Combination on Blood Glucose and Hba1c Levels in HIV-Protease Inhibitor Treated *Rattus Norvegicus*

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ABSTRACT

Available reports show that medication with HIV protease inhibitors (HIV-PIs) can lead to high blood glucose in HIV positive patients. There is therefore a need to find ways of mitigating this hyperglycemic effect as a result of treatment with HIV-PIs. We assessed the effect of administration of the flavonoids Diosmin and Hesperidin on HIV protease inhibitor (HIV-PIs) induced elevated blood glucose and HbA1c levels in Rattus norvegicus albinus (wistar rats). Seventy- two Wistar rats were randomly assigned into six groups of twelve rats each. Group 1 was a Control. The other five groups (2-6) were treated with LPT/RTV(2/0.5mg/kg), ATV/RTV(3mg/1 mg/kg), LPV/RTV plus DIOS/HES(2/0.5 mg /kg plus 4.5/0.5 mg/kg), ATV/RTV plus DIOS/HES(3/1mg per kg plus 4.5/0.5 mg /kg) and DIOS/HES(4.5/0.5mg/kg) respectively administered twelve hourly. The blood glucose and HbA1c concentration were measured, analyzed and presented using descriptive statistics and one way ANOVA. The Control group, DIOS/HES and the ATV/RTV+DIOS/HES treated groups did not show any significant differences in their group mean blood glucose levels from the second week of treatment to the end of the study. The ATV/RTV (5.95±2.15 mmol/L on week 3), LPV/RTV (5.14±0.92 mmol/L on week 3) and LPV/RTV+DIOS/HES (5.30±1.72 mmol/L on week 3) treated groups had significantly elevated group mean blood glucose levels on the third week of the study compared to the control group (3.88±0.42 mmol/L. The HIV-protease inhibitors increased group mean HbA1c levels with the LPV/RTV treated group increasing from 4.67± 0.16 percent on day 0 to 5.45±0.77 percent on day 42 ($p<0.01$) and ATV/RTV treated group mean HbA1c level increasing from 4.52±0.16 percent on day 0 to 5.13±0.53 percent on day 42 ($p<0.01$) while the DIOS/HES group mean HbA1c levels decreased significantly (4.73±0.16 percent at day 0 to 3.35±0.48 percent on day 42, $p<0.001$). The results demonstrated that the co-administration of ATV/RTV with DIOS/HES significantly reduced the blood glucose elevating effects of the ATV/RTV combination. The co-administration of the flavonoids with HIV-PIs also reduced the blood HbA1c levels. We recommend further studies on the effect Hesperidin and Diosmin on blood glucose and HbA1c in humans on HIV-PIs.

Keywords: HIV, HIV-Protease inhibitors, , Blood glucose, HbA1c, Diosmin, Hesperidin

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INTRODUCTION

HIV- protease inhibitors (HIV-PIs) are key drugs in HAART in the management of HIV/AIDS patients and have led to a remarkable decrease in mortality and morbidity amongst HIV/AIDS patients. However, they are now generally known to cause a number of side effects such as lipodystrophy, lipoatrophy, and hyperlipidemia and insulin resistance leading to glucose intolerance, hyperglycemia and altered plasma lipid profiles. Hyperlipidemia is characterized by increased triglycerides and fatty acid plasma levels (Palella et al., 1998; Paterson et al., 2000). Lipodystrophy is the abnormal distribution of body fat characterized by truncal obesity and a buffalo hump. Lipoatrophy is fat wasting particularly from the face and the limps, and is characterized by decreased leptin levels in blood (Baril et al., 2005; Carr et al., 1998; Estrada et al., 2006; Koutkia & Grinspoon, 2004). Insulin resistance and the accompanying hyperglycemia in patients on HIV Protease Inhibitors is a syndrome of elevated insulin levels which is however ineffective in reducing blood glucose levels. This is due to interference with the signal transduction pathway in the target cells particularly in those involving the glucose transporters (GLUT-4) in skeletal muscle and adipose tissue (Calderhead et al., 1990; Cheatham et al., 1996; Kozka et al., 1991; Murata et al., 2000, 2002).

This study is important in the sense that as of 2023, worldwide 30.7 million HIV/AIDS patients are on highly active antiretroviral therapy (HAART) including 1.34million Kenyans (Global HIV & AIDS Statistics — Fact Sheet | UNAIDS, n.d.; HIV – Reported Number of People Receiving Antiretroviral Therapy, n.d.). HIV- protease inhibitors (PIs) which form an important pillar of HAART can precipitate glucose intolerance leading to chronic hyperglycemia which in turn can result to overt diabetes mellitus (Carr et al., 1998). Any effort that can generate new ways of countering these adverse effects without producing more complications and adverse effects is worthwhile. This study was designed to establish if the flavonoids Diosmin and Hesperidin (naturally occurring and safe) together when co-administered with Protease Inhibitors can reduce these adverse effects.

Hesperidin and Diosmin belongs to a class of flavonoids called flavanones which can be obtained from citrus fruits (Vinayagam & Xu, 2015). These compounds have been found to exhibit blood sugar level lowering effects by various cellular and molecular mechanisms. They are thought to act by their antioxidant action, which action is protective to β-cells of the pancreatic islets of Langerhans. There is evidence that they scavenge free radicals thus reducing oxidative stress on the β-cells resulting in increased insulin secretion (Coskun et al., 2005; Vessal et al., 2003). In a nutshell flavonoids also promote the peripheral utilization of glucose (increase sensitivity of cells to insulin), slow glucose absorption from the small intestine and enhance insulin secretion (Jadhav & Puchchakayala, 2012). Flavonoids also act by targeting molecular pathways in the β-cells, hepatocytes, adipocytes and skeletal muscles cells. These compounds also reduce apoptosis of the β-cells by their antioxidant action (Oladele et al., 2010). The main aim of this study was to assess if this adverse effect – hyperglycemia, induced by HIV- protease inhibitors due to glucose intolerance can be ameliorated by the co-administration of flavonoids – specifically the Diosmin /Hesperidin combination together with the HIV protease inhibitors - Lopinanvir/Ritonavir combination and Atazanavir/Ritonavir combination.

So far, studies done have focused separately on either the hyperglycemic effects of HIV-Protease inhibitors or the antihyperglycemic effects of flavonoids on diabetic subjects. For instance in a study done in Taiwan (Hsu et al., 2017), Diosmin was demonstrated to alleviate hyperglycemia in type 1 diabetic rats. A similar study in Japan (Akiyama et al., 2009) also demonstrated the antihyperglycemic effects of hesperidin on rats. In 2004, (Schütt et al., 2004), workers in Germany confirmed that chronic exposure of Beta cells of langerhans to HIV-protease inhibitors led to hyperglycemia as a result of development of insulin resistance. A study in India (Marella, 2017), emphasized that it is now a recognized fact that flavonoids are potent antidiabetic agents. A case report from Nigeria (Bakari et al., 2007) clearly shows how antiretroviral therapy in a patient led to the development of diabetes mellitus in that patient. Researchers in India have demonstrated that diabetes mellitus can be one of the consequences of HAART (Kalra et al., 2011). However so far, no study has assessed the effect of Hesperidin and Diosmin on HIV- Protease Inhibitor induced hyperglycemia. The presence of this knowledge gap formed the basis and the justification of this study.

This study was limited to assessing the effect of two of the most commonly used HIV-Protease Inhibitor Combination in Kenya (Lopinavir+Ritonavir and Atazanavir+Ritonavir) on blood glucose levels and HbA1c levels of Wistar rats. The study also assessed the effect of the flavonoid combination of Hesperidin (HES) and Diosmin (DIOS) on the hyperglycemia induced by these HIV-Protease Inhibitors on Wistar rats. Wistar rats were used as an animal model because they are easily available, easy to handle and metabolically handle glucose and other drugs in ways similar to humans (Lindsey & Baker, 2006; Wistar Rat - an Overview | ScienceDirect Topics, n.d.).

METHODS

Study Location & Design

This study that was conducted at the zoology Laboratories of the University of Eldoret in Uashin Gishu County, Kenya. This was a randomized controlled experimental study in

which seventy- two Wistar rats were randomly assigned into six groups of twelve rats each. Each group was subjected to a specific treatment as described under treatments (See table 1).

Sampling procedure

Seventy-two Wistarrats, aged 3-4 months weighing around 150g – 200g were randomly selected. Twelve animals were then randomly assigned into the six different treatment groups.

Treatment protocols

The rats were acquired and kept for two weeks to acclimatize in their new environment at the university of Eldoret zoology laboratories. Each animal was weighed and tagged with a serial number ranging from 1 to 12 for each group and labeled appropriately. Their baseline random blood glucose was measured by glucometer and recorded. Any animal that was found to have a random, blood glucose levels of more than 7mmol/L was retested and if confirmed was excluded from the procedure and replaced. This parameter was used as a threshold for diagnosing hyperglycemia. The animals were then randomly assigned into 6 groups (using simple random sampling and labelled as stated above) and placed into separate cages labelled and partitioned appropriately. The HIV-PIs and HES/DIOS were administered to the animals by the oral route using a gastric gavage syringe at doses indicated in Table 1. The animals were fed on rat pellets (regular diet) and given enough water to drink.

Table 1:

Treatment protocols summary

Group	Treatment
Group1	Rat food pellets only.
Group2	Lopinavir/Ritonavir(2/0.5mg/kg body weight) 12 hourly.
Group3	Atazanavir+Ritonavir(3mg/1 mg/kg body weight) 12 hourly.
Group4	Lopinavir/Ritonavir + Hesperidin/Diosmin (LPV/RTV: 2/0.5 mg /kg body weight plus HES/DIOS: 4.5/0.5 mg/kg body weight) 12 hourly.
Group5	Atazanavir/Ritonavir + Hesperidin/Diosmin(ATV/RTV: 3/1mg per kg plus HES/DIOS: 4.5/0.5 mg /kg) 12 hourly.
Group6	Hesperidin/Diosmin (4.5/0.5mg/kg body weight) 12 hourly.

Hesperidin-Diosmin combination

Daflon™ Tablets (contains Hesperidin 450mg and Diosmin 50mg) were used as source of hesperidin and diosmin. They were sourced locally from appointed distributors.

Lopinavir/Ritonavir and Atazanavir/Ritonavir combinations

Lopinavir/Ritonavir (200/50mg) combination tablets and Atazanavir/Ritonavir (300/100 mg) tablets were sourced locally from appointed distributors.

Data collection

Blood glucose

The blood glucose levels were taken at the beginning of the study before the treatments were administered (baseline) and thereafter the blood glucose levels were measured once a week on the same day and time over the duration of the study (6 Weeks) and the results were recorded and stored in password secured spread sheets. On day 1, blood glucose was measured using blood obtained by tail prick of the rat tail vein and glucometer. On day7, day14, day21, day28, day35 and day42 each rat was taken in turn, according to its label and blood was drawn from its tail vein and blood glucose level measured using the glucometer and recorded. The blood glucose levels were measured using a glucometer (On - Call plus™ by ACON laboratories Inc., 1025 Mesa Rim Road, San Diego, CA 92121, USA, 2016 model).

HbA1c levels

At the start of the study, blood samples from the tail vein of the animals were collected into

specimen tubes with an anticoagulant (EDTA) to keep the specimen uncoagulated. The blood sample was taken using a pipette and placed in the sample cartridge of the MISIPA-i3™ Cartridge Based Protein Auto-Analyzer (AGAPPE DIAGNOSTICS™, Switzerland GmbH). Eight minutes after loading, the results were printed and the HbA1c values in percentage were recorded. At the end of the study (Day 42) the animals were sacrificed using chloroform to induce anesthesia and blood samples were collected by cardiopuncture into specimen tubes with anticoagulant and the above procedure of HbA1c assay was repeated.

Data analysis

The data was summarized using the mean± SD (standard deviation) and displayed in tables and graphs to demonstrate the main trends of blood glucose (mmol/L) and percentage blood HbA1c levels. Inferential statistical analysis of the group mean blood glucose and percentage blood HbA1c was done using one-way ANOVA (F-value). Differences between the group means of these measurements due to experimental treatments was assessed by computation of the Least Significant Difference (LSD) through ad hoc multiple group comparisons. The associated p-values of the differences of the group means and the effect sizes of the F-values (partial eta squared, η^2) were noted. Results were considered significant if the test statistics (F-value and LSDs) had p –values less than or equal to the level of significance i.e. α=0.05 (P≤0.05). The effect size of the test statistics was interpreted using the Cohen's Guidelines (Cohen, 2013)

Ethical considerations

This study involved the use of animals - albino Wistar rats (*Rattus norvegiccus albinus*). Permission to use the animals as the experimental model was obtained from the ethical review board on the use of laboratory animals from The University of East Africa, Baraton, Kenya (Permit number B92272019).

RESULTS

Blood glucose levels in HIV- protease inhibitor treated rats

The baseline group mean blood glucose levels were measured before the treatments were administered with the control, LPV/RTV and ATV/RTV groups attaining mean group blood glucose levels of4.07±0.27, 4.18±0.25 and 4.09±0.37 mmol/L respectively (figure 1). These differences were not statistically significant (p=0.113, η^2=0.124).

By the end of the third week of treatment, the groups treated with ATV/RTV and LPV/RTV attained highest group mean blood glucose levels of 5.95±2.15 mmol/L and 5.14±0.92 mmol/L respectively, compared to the Control (3.88+/-0.42 mmol/L) indicating a highly significant difference in the group mean blood glucose levels between these treatments (p<0.0001, η^2=0.272) and that over 27% of the group difference was due to the treatments (effect size). At the end of the fourth week, the ATV/RTV treated group had the highest group mean (4.91±0.90 mmol/L) followed by the LPV/RTV treated group (4.64±0.29 mmol/L) and compared to the control group (4.11+/-0.25 mmol/L). By the end of the fifth week the ATV/RTV treated group still had the highest group mean blood glucose level (5.03±0.63 mmol/L) followed by the LPV/RTV treated group (4.51±0.60 mmol/L) and the control (4.16+/-0.38 mmol/L) respectively. The difference between their group mean blood glucose levels was significant (p<0.0001, η^2=0.349). At the end of the sixth week, the ATV/RTV treated group had higher group mean blood glucose level (4.89±0.71 mmol/L) followed by the LPV/RTV treated group (4.43±0.77 mmol/L) with the control attaining 3.94±0.32mmol/L. These group mean blood glucose levels were significantly different (p=0.03, η^2=0.166).

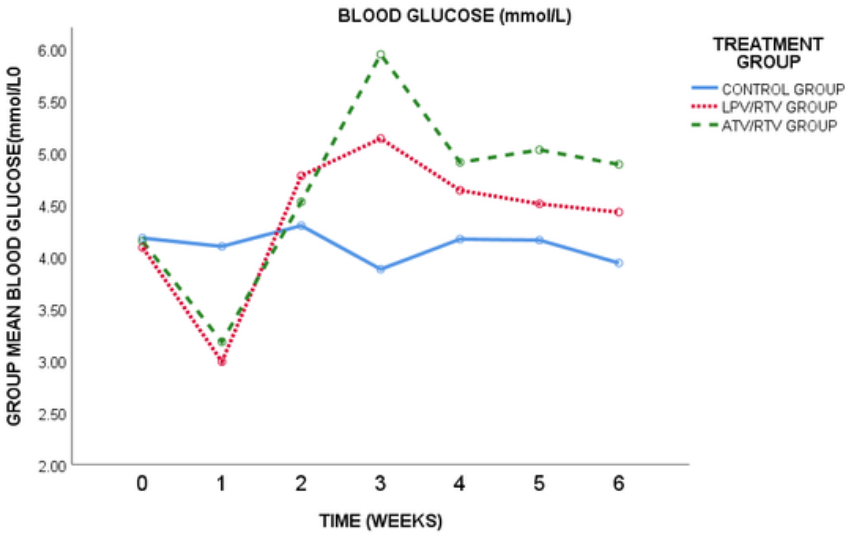

Figure 1:
Mean Blood Glucose Levels of HIV-Protease Lopinavir /Ritonavir (LPV/RTV) and Atazanavir/Ritonavir (ATV/RTV) Treated Groups as a Function of Time.

The Blood Glucose Levels of the HIV-PI Plus Diosmin/Hesperidin Treated Groups

At the end of the second week of treatment the group blood glucose mean of the LPV/RTV (4.78±0.65 mmol/L) treated group was higher compared to the group treated with LPV/RTV+DIOS/HES (4.32± .33mmol/L) and the Control group (4.30±0.47 mmol/L).The differences were as follows: the control group versus LPV/RTV treated group (p=0.006), the control group versus the LPV/RTV+DIOS/HES treated group (p=0.55) and the LPV/RTV versus LPV/RTV+DIOS/HES (p=0.028).

By the end of the third week of treatment, the Control (3.88+/-0.42 mmol/L) and the LPV/RTV (5.14+/-0.92 mmol/L) groups had significant differences in their group mean blood glucose concentrations (diff.=1.26 mmol/L, p=0.016). The Control group and the LPV /RTV + DIOS /HES(5.29+/-0.1.72 mmol/L) treated groups also had a significant difference in their group mean blood glucose levels (diff.=1.41 mmol/L, p =0.007) with the LPV/RTV+DIOS/HES group attaining the highest blood glucose mean among the three groups at this point in time (Figure2).

By the end of the fifth week the Control (3.94±0.77 mmol/L) and the LPV/RTV (4.43±0.77 mmol/L) treated groups had a significant difference in the group mean blood glucose (0.492 mmol/L, p=0.043). The LPV/RTV+DIOS/HES treated group had a higher mean blood glucose than the Control group (difference=0.840 mmol/L, p<0.0001.

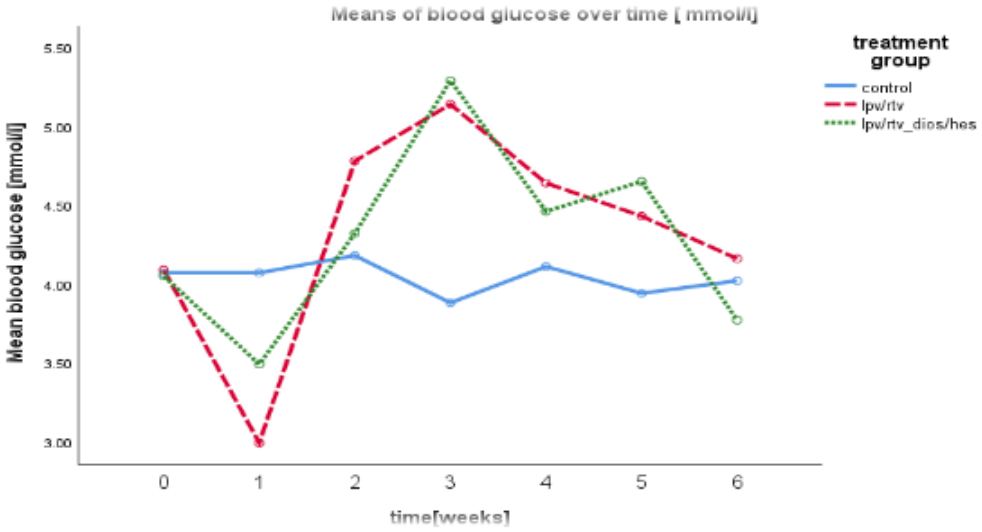

Figure 2:
The Group Mean Blood Glucose of the Control, the Lopinavir/Ritonavir (LPV/RTV) and the Lopinavir/Ritonavir+Diosmin/Hesperidin (LPV/RTV+DIOS/HES) Treated Groups Over Time.

The blood glucose levels in Atazanavir/Ritonavir and Atazanavir / Ritonavir plus Diosmin/Hesperidin treated rats

At the inception of the study (day 0) there was no significant difference in the group mean blood glucose of the control (4.07±0.27mmol/L) group, the ATV/RTV9 (4.07±0.36 mmol/L) and the ATV/RTV+DIOS/HES (4.09±0.28 mmol/L) groups

By the end of the third week, the ATV/RTV treated group showed a drastic increase in the mean blood glucose (5.95±2.15 mmol/L) compared to the control group (3.88±0.42 mmol/L) and had a highly significant difference (diff.=2.08 mmol/L, p< 0.001). There was however no significant difference in the group mean blood glucose level between the control group and the ATV/RTV+ DIOS/HES (4.45±0.79 mmol/L) treated group (diff.=0.57 mmol/L, p=0.277). There was also a significant difference in the group mean blood glucose levels between the ATV/RTV and the ATV/RTV + DIOS/HES treated groups (diff.=1.50 mmol/L, p= 0.006). On day 28 (end of the 4th week) the ATV/RTV treated group had a higher mean blood glucose level (4.91±0.91 mmol/L) than the control group (4.11±0.25 mmol/L) with a significant difference (diff.=0.80 mmol/L, p=0.002) confirming that this HIV-protease inhibitor (ATV/RTV) increased blood glucose levels. The Control Group and the ATV/RTV+DIOS/HES (4.22±0.94 mmol/L) treated group did not have significantly different levels of group mean blood glucose (diff.=0.110 mm0l/L, p = 0.668) showing that the addition of DIOS/HES to ATV/RTV reduced the hyperglycemic effects of ATV/RTV administration to the animals. At this point in time the ATV/RTV and ATV/RTV+ DIOS/HES treated groups demonstrated a significant difference in their blood glucose (diff.=0.69 mmol/L, p = 0.009). At the end of week five, the Control Group (3.94+/-0.32 mmol/L) and the group treated with ATV/RTV (4.89+/-0.71 mmol/L) had a highly significant difference (diff.=0.950 mmol/L, p<0.0001). The Control Group and the ATV/RTV+DIOS/HES (3.84±0.65 mmol/L) treated group did not have a significant difference in their blood glucose (diff.=0.395 mmol/L, p = 0.084). The ATV/RTV group had a higher group mean blood glucose (4.89±0.71 mmol/L) than the ATV/RTV+DIOS/HES treated group (4.34±0.42 mmol/L), (diff.=0.56 mmol/L, p=0.019).

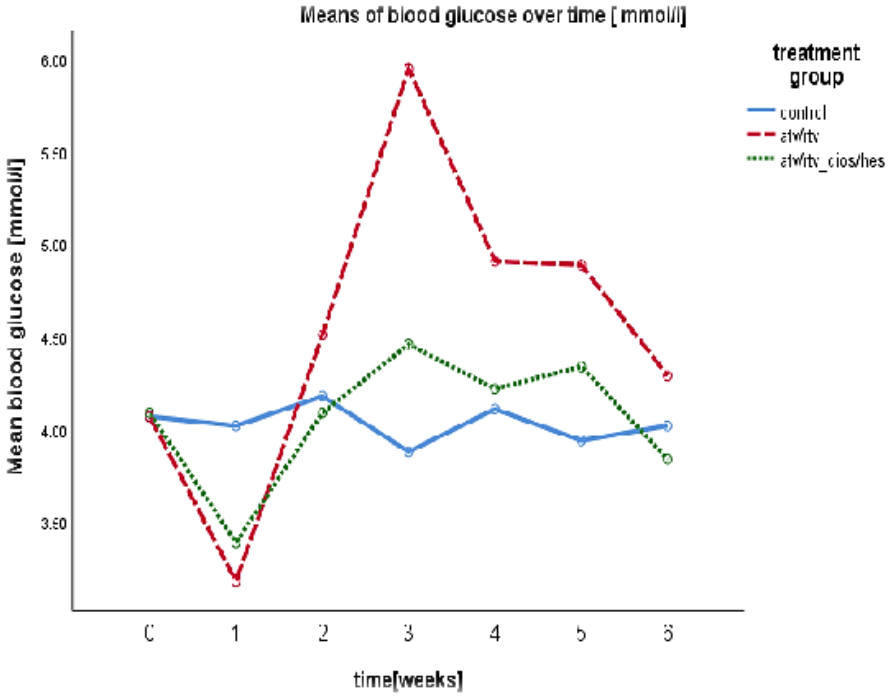

Figure 3:
The Group Mean Blood Glucose of the Control, Atazanavir /Ritonavir (ATV/RTV) and Atazanavir /Ritonavir+Diosmin/Hesperidin (ATV/RTV+DIOS/HES) Treated Groups.

Blood Glucose Levels of the Diosmin /Hesperidin, the ATV / RTV + DIOS/HES and LPV/RTV + DIOS/HES Treated Rats

The control and the DIOS/HES treated groups did not exhibit any significant differences in their group mean blood glucose levels for the entire duration of the study. The control group (4.02±0.25 mmol/L) and ATV/RTV+DIOS/HES (3.39±0.48 mmol/L) had a significant difference (p=0.014) only once at the end of the first week of treatment. From the second week of treatment, the control, the DIOS/HES and the ATV/RTV + DIOS/HES treated groups did not exhibit any significant difference in their group mean blood glucose levels (figure 4).

The Control Group and the LPV/RTV+DIOS/HES (3.49±0.63 mmol/L) had a significant difference (diff.=0.53mmol/L, p=0.038) at the end of the first week of treatment. At the end of the third week, the Control Group (3.88±0.42 mmol/L) and the LPV/RTV+DIOS/HES (5.29±1.72 mmol/L) had a significant difference (diff.=1.41mmmol/L, p=0.007) and on week five the LPV/RTV+DIOS/HES (4.78±0.64 mmol/L) and the control (3.94±0.32 mmol/L) had a highly significant difference (diff.=0.84mmol/L, p<0.0001). The LPV/RTV+DIOS/HES and ATV/RTV+DIOS/HES treated groups did not show any significant differences throughout the entire duration of the study.

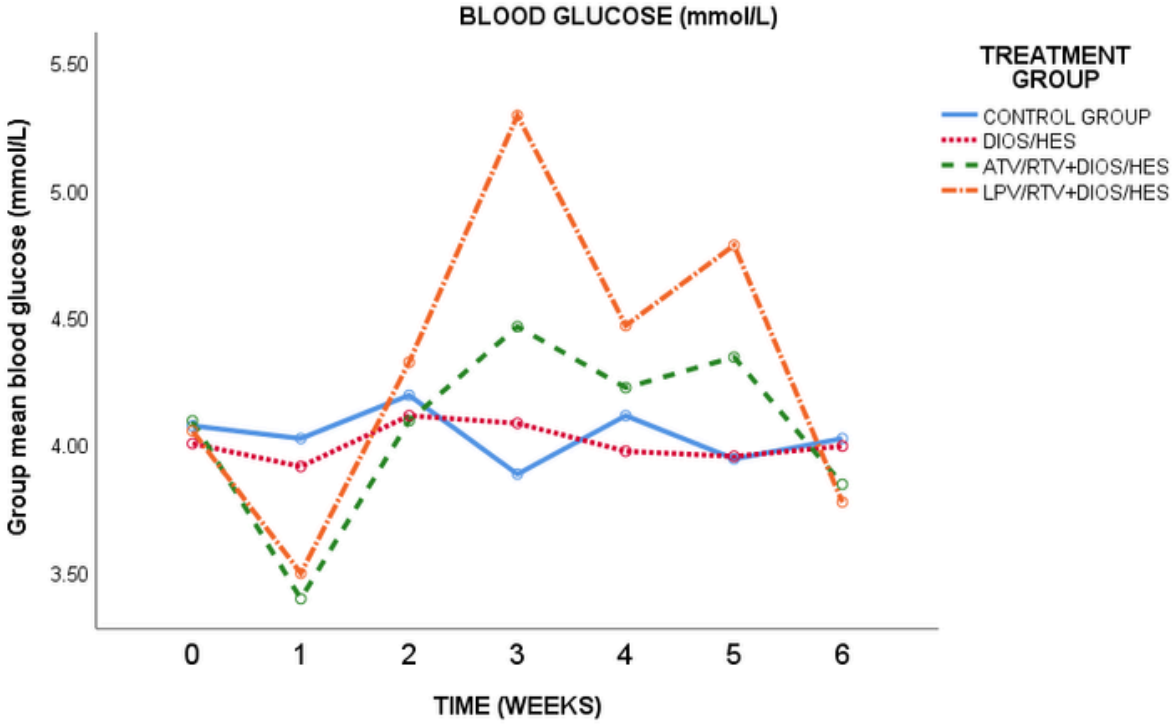

Figure 4:
Group Mean Blood Glucose Levels of The Control, Lopinavir/Ritonavir+Diosmin / Hesperidin (LPV/RTV + DIOS/HES), Atazanavir/Ritonavir+Diosmin/Heseridin (ATV /RTV +DIOS/HES) and the Diosmin/ Hesperidn (DIOS/HES) Treated Rats.

Blood glucose levels of diosmin/hesperidin treated rats

The mean blood glucose levels of the Diosmin/Hesperidin (DIOS/HES) treated group did not show any significant difference from the control group throughout the duration of the study (Table 2).

Table 2:
Blood Glucose Levels (mmol/L) of the Control and DIOS/HES Groups

Time (Weeks)	Control group(mmol/L)	Diosmin/Hesperidin Group(mmol/L)	P - value
Baseline (Day 0)	4.07±0.27 n=12	4.00±0.35 n=12	0.612
Week 1	4.02±0.25 n =12	3.91±0.32 n=12	0.663
Week 2	4.19±0.32 n=12	4.11±0.43 n = 12	0.690
Week 3	3.88±0.42 n=12	4.07±0.26 n=12	0.277
Week 4	4.11±0.25 n=12	3.97±0.24 n=12	0.572
Week 5	3.94±0.32 n=12	3.95±0.18 n=12	0.970
Week 6	4.02±0.37 n=12	3.99±0.21 n=12	0.884

HbA1c Levels Before and After Treatment With LPV/RTV, ATV/RTV) and Diosmin/Hesperidin

Atbaseline the blood from the animals did not show any significant difference in group mean HbA1c levels ($F_{1-\alpha/2}$ (5, 66) = 0.658 at α=0.05, p=0.656, η^2=0.048).At the end of the treatment period of 6 weeks (42nd day) there was a clear and significant difference among the groups in terms of their mean HbA1c percentage as shown by a one -way ANOVA ($F_{1-\alpha/2}$ (5, 60) of 20.52 (p<0.001) with a partial η^2 =0.635). A partial eta squared of 0.635 is a large effect size (Cohen, 1988) and demonstrates that the treatments accounted for 63.5% of the observed difference in the group mean HbA1c levels. It was observed that the administration of Diosmin /Hesperidin combination alone (group 6) produced the biggest decrease in the group mean percentage HbA1c over the duration of treatment (4.71±0.55% to 3.35±0.47%). On the other hand, the groups administered with Lopinavir/Ritonavir combination (4.67±0.67% to 5.45±0.77%) and Atazanavir/Ritonavir (4.52±0.62% to 5.13±0.53%) exhibited a significant increase in the percentage HbA1c (Figure5). At the end of the six weeks (day 42) the control group had a significantly higher mean HbA1c level than the ATV/RTV+DIOS/HES treated group (diff.=1.03%. p<0.0001) and the DIOS/HES treated group (diff.=1.62%, p<0.0001)

The LPV/RTV treated group also had higher group mean percentage HbA1c levels than the DIOS/HES treated group (diff.=2.18%, p<0.0001, figure 5). The ATV/RTV treated group had higher group mean HbA1c levels than the ATV/RTV+DIOS/HES treated group (diff.=1.2%, p<0.0001) whereas the LPV/RTV+DIOS/HES treated group had higher HbA1c levels than the ATV/RTV+DIOS/HES treated group (diff.=0.96%, p<0.0001) and the DIOS/HES treated group (diff.=1.55%, p<0.0001) respectively. Compared to all the other groups the DIOS/HES treated group (group 6) had the lowest level of percentage blood HbA1c (3.35±0.55%,Figure 5) indicating that the administration of DIOS/HES had a significant effect of lowering the blood HbA1c levels in the experimental rats.

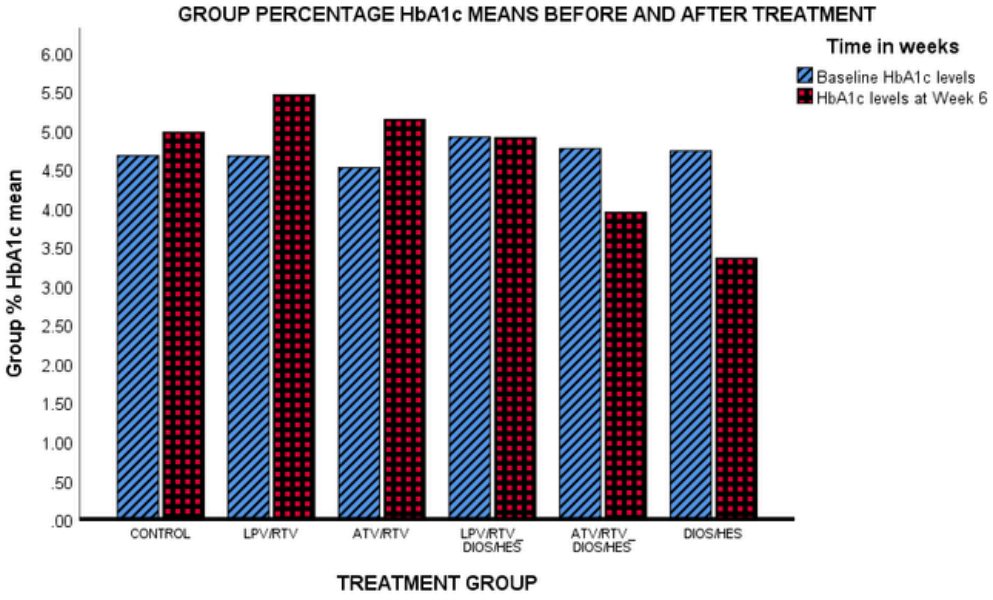

Figure 5:
Group % HbA1c Mean Before and After Treatment With HIV-PIs

DISCUSSION

The mean blood glucose of the groups treated with lopinavir/ritonavir or atazanavir/ritonavir was generally higher than that of the control and the Diosmin/Hesperidin treated groups. The mechanisms by which the HIV-protease Inhibitors (HIV-PIs) increase blood glucose include inhibition of insulin stimulated glucose uptake by cells like adipocytes and skeletal muscle cells. HIV-PIs are also thought to interfere with insulin signal transduction mechanisms that lead to the translocation of for example the GLUT4 transporters to the cell surface to facilitate glucose uptake by cells. Some of these HIV-PIs also induce insulin resistance. They also cause impairment in insulin secretion while stimulating endogenous glucose production (gluconeogenesis). One of the consequences in the increase in blood glucose in patients on HIV- protease inhibitors is that it may sometimes result in overt diabetes mellitus (Barbaro, 2005; Barth et al., 2010; Carr et al., 1999; Dever et al., 2000; Reust, 2011; Salehian et al., 2005; Zeldin & Petruschke, 2004). This can result in complicating patient management because the patient may have to take more drugs to manage diabetes mellitus and this can lead to an increase in the pill (medicines) burden given that most of them are already on ARVs (HAART). Atazanavir/Ritonavir caused a higher increase in the blood glucose compared to Lopinavir/Ritonavir (Aberg et al., 2012; Noor et al., 2006; Stanley et al., 2009). The results of this study are line with previous findings that some of the HIV-PIs can cause an increase in blood glucose levels by causing oxidative stress that may result in increased β-cell apoptosis leading to decreased insulin secretion (Carr et al., 2008; Chandra et al., 2009; Dubé, 2000; Flint et al., 2009; Howard et al., 2005; Hruz et al., 2008; Hui, 2003; Lee et al., 2005; Lien & Feinglos, 2005; Murata et al., 2000; Noor et al., 2004; Sangraula et al., 2001; Schütt et al., 2004; Tsiodras et al., 2000; Viganò et al., 2009; Woerle et al., 2003).

These results demonstrate that the co-administration of DIOS/HES with HIV-protease inhibitors had a bigger impact in terms of the reduction of the group mean blood glucose level elevation caused by treatment with ATV/RTV compared to the impact on the blood sugar elevation caused by treatment with LPV/RTV. The differences were even higher between the DIOS/HES combination treated and the LPV/RTV and ATV/RTV combination treated groups. During the duration of the study, the animals treated with LPV/RTV had a significant group mean blood glucose level difference with the group treated with DIOS/HES combination. The group on ATV/RTV and the group treated with DIOS/HES demonstrated significant differences in their mean blood glucose levels during most of the duration of the study. The addition of Diosmin/Hesperidin combination to the Atazanavir /Ritonavir treated groups had a significant effect of reducing the blood glucose levels. This is an indication that addition of the Flavonoids (Diosmin/Hesperidin Combination) had a significant group mean blood glucose

stabilizing effect to the extent that it was more or less similar to that of the Control group. On the other hand the Diosmin/Hesperidin combination did not have a significant effect on the blood glucose levels of the Lopinavir/Ritonavir treated group which remained more or less elevated (Constantin et al., 2014; El-Marasy et al., 2014; Sahnoun et al., 2017). When administered to untreated animals (those not on HIV-PIs) the Diosmin/Hesperidin Combination did not have a significant impact on blood glucose levels. These findings also confirm previous findings by other researchers that flavonoids have an antihyperglycemic effect(Jadhav & Puchchakayala, 2012) (Tapas et al., 2008).

HbA1c is glycated hemoglobin. It is the hemoglobin formed by the unregulated (no enzymatic involvement) reaction between hemoglobin and blood glucose. The glucose reacts with the β-chain of hemoglobin. The levels of HbA1c in blood is dependent on the levels of blood glucose (Florkowski, 2013; Sherwani et al., 2016; Trial, 2002).The elevated levels of blood HbA1c are a reflection of chronic hyperglycemia (Rohlfing et al., 2002; Vos et al., 2012). Since the formation of HbA1c is a non-enzymatic reaction, it is mainly driven by the law of mass action (Higgins, 2012b; Ładyżyński et al., 2011; Sen et al., 2005). HbA1c is one of the advanced glycation end products (AGEs) – the products formed by chronic hyperglycemia. Elevated levels of HbA1c leads to a number of adverse effects such as microvascular complications that lead to increased thrombogenicity of blood and poor wound healing in case of injuries like diabetic foot, increased levels of glucose may result in glucotoxicity to the β-cells of the pancreas and increased HbA1c levels may increase the risk developing coronary heart disease and stroke (Lind et al., 2009; Osende et al., 2001; Sherwani et al., 2016; Škrha et al., 2016).

Such an increase in the risk of developing these complications in HIV infected patients can lead to an increase in the need for more medications (to control hyperglycemia and treat complications of elevated HbA1c) on top the ones these patients are already taking (ARVs) to prevent the progression of HIV infection to full blown AIDS. This may increase the pill (number of medicines) burden and the associated adverse effects. In this study the treatment of the animals with HIV-Protease inhibitors led to an increase in the blood HbA1c levels in both the LPV/RTV and the ATV/RTV treated groups. The co-administration of the flavonoids DIOS/HES combination with the HIV-Protease inhibitors ATV/RTV led to a significant reduction in the blood HbA1c levels. The group treated with the flavonoid combination of Diosmin and Hesperidin showed the largest decrease in blood HbA1c levels. The group mean HbA1c level of this group was the lowest at the end of the study.

These findings show that these drugs (HIV-protease inhibitors) generally increased the blood glucose levels and this increased blood glucose led to the glycation of hemoglobin leading to the observed increase in the percentage HbA1c levels. This is because the levels of HbA1c are directly proportional to blood glucose levels and its generation is generally driven by the law of mass action. HbA1c levels are strongly associated with some of the end stage complications of DM (Farmer et al., 2007; Higgins, 2012a; Ittle, 2002; Khattab et al., 2010; Petitti et al., 2009; Sherwani et al., 2016; Stolar, 2010). This finding is consistent with those of other researchers who have established that the administration of flavonoids such Diosmin and Hesperidin to human and animal subjects tends to reduce the level of advanced glycated end-products (AGEs) including HbA1c in blood (Ahmad et al., 2013; Pashikanti et al., 2010).

CONCLUSION

In conclusion the present study found that treatment of the rats with the HIV-PIs (ATV/RTV or LPV/RTV) increased the blood glucose levels. The administration of DIOS/HES combination the on rats (Rattus norvegicus albinus) treated with HIV-PIs led to a significant decrease in the group mean blood glucose levels in the ATV/RTV treated group but not in the treated LPV/RTV group. The co-administration of DIOS/HES with the ATV/RTV combination (a HIV-PI combination) led to significant reduction in the HbA1c levels. We recommend studies in human subjects to further explore these findings.

Declaration of Conflict

The authors have no conflict of interest to declare. This study was wholly funded by the authors.

Role of the Authors

Akunga N G – Concept, development, Data collection and analysis
Ngw'ena G A M – Concept development and manuscript preparation.
Owiti O M G – Manuscript preparation and editing.

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