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# Histomorphological and biochemical changes in the liver tissue following adjuvant treatment with *hypoxis hemerocallidea* and Antiretroviral Drugs in diabetic rats

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**Abstract**

**Introduction:** Highly Active Antiretroviral Therapy (HAART) has been used in the management of people living with Human immunodeficiency virus (HIV). However, the long-term effects cause diabetes and result in liver damage. Similarly, *Hypoxis hemerocellidae* (*H.h*) has been used traditionally to treat diseases including HIV and Diabetes. The study aimed to investigate the effects of aqueous extract of H.h on the liver cytoarchitectonic in diabetic experimental animals under antiretroviral therapy.

**Methods**: Thirty-six (36) adult male Sprague-Dawley rats were used and divided into 6 groups namely: A ( control), B (diabetic) received distilled water, C (diabetic + 50mg/kg, *H.h*), D (diabetic + HAART), E ( diabetic + HAART+ 250 mg/kg VIt C), F (diabetic + HAART + 50mg/kg of *H.h*). Blood glucose levels, Body weight, oxidative stress markers and liver histomorphology of the experimental animals were measured and analyzed.

**Results:** The blood glucose levels of animals administered with *H.h* were significantly reduced compared with diabetic control. Group E had significantly reduced blood glucose levels compared with other treated groups D and F. There is a significant reduction of AST in group C and E compared to other groups D and F. Group F showed a significant reduction in MDA and an improvement in GSH compared to other treated groups except group E. Histologically, H& E and PAS staining revealed an improvement in groups C and F compared to the diabetic-treated groups.

**Conclusion:** This study demonstrated that *H.hemerocallidae* mitigated the metabolic effect of HAART in diabetic rats. Still, the antidiabetic and antioxidant effects, in combination with antiretroviral therapy need further investigation at different doses.

**Keywords**: *Hypoxis hemerocallidea*, HIV and HAART, Diabetes Mellitus, Liver

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## Introduction

The Human Immunodeficiency Virus (HIV) and acquired immunodeficiency syndrome (AIDS) pandemic remains the biggest socio-economic challenge that is facing the world at large, affecting mostly the young and economically productive population(Drimie et al., 2002). Despite the availability of various anti-viral medications, the need for new antiretroviral agents continues to increase because the existing medications do not provide a complete curation and cause serious side effects(Geronikaki et al., 2016). Many HIV/AIDS patients receiving fixed-dose combination drugs: Efavirenz (EFV), Emtricitabine (FTC), and Tenofovir disoproxil fumarate (TDF) become glucose intolerant and insulin resistant, leading to diabetes mellitus associated with liver damages(Morse & Kovacs, 2006). Type 2 diabetes mellitus (T2DM) associated with long-term use of combined antiretroviral therapy (cART) has since become a major cause of morbidity and mortality. More so, diabetes mellitus and cART long-term use raise the risks of non-alcoholic liver disease(Mohamed et al., 2016; Soriano et al., 2008).

In parts of Africa continent including South Africa, the use of herbal medicine for the management of HIV is common due to the high-cost effectiveness of providing HIV treatment(Kankara et al., 2022). Recently, the South African government promoted the use of *H. hemerocallidea* (commonly called African potato) for the treatment of HIV and associated symptoms(Ismail et al., 2017; Mills et al., 2005). The herbs of *H. hemerocallidea* have long been used by Africans as a traditional medical treatment for various diseases and have been the subject of several scientific studies(Mlilo & Sibanda, 2022).

The medical plant *H. hemerocallidea* has been reported to boost the immune system of HIV-positive patients (Matyanga et al., 2020). The extract of *H. hemerocallidea* was reported to reduce high blood pressure, prostate hypertrophy, adult-onset diabetes, and HIV/AIDS-related conditions(Ojewole, 2006; Rahman et al., 2022). Biomedical studies have revealed that the extracts possess anti-inflammatory, antineoplastic, antioxidant, antidiabetic, and anti-viral properties in vivo and in vitro(Olasile et al., 2018). Hypoxoside is an active phytochemical component of *H. hemerocallidea* converted by the action of $\beta$ glucosidase enzyme to Rooperol which has potent pharmacological properties relevant to cancer, inflammations, and HIV(Olarewaju et al., 2022).

Another study also reported that H. *hemerocallidea* significantly reduced serum cholesterol and improved liver fat(Hovenkamp et al., 2008).

As a result of these claims, there is paucity in the literature suggesting the effects of *H. hemerocallidea* might attenuate the toxicities of the liver associated with HAART and diabetes.

Thus, this study aimed to investigate the potential effects of *Hypoxis Hemerocallidea* on the cytoarchitectonic properties of the liver in Streptozotocin-induced diabetic Rats treated with antiretroviral therapy.

## Materials and methods

### Drugs

A single dose of Odimune/HAART (containing efavirenz (EFV) 600 mg, emtricitabine (FTC) 200 mg, and Tenofovir (TDF) 300 mg) was obtained from Pharmed Ltd., Durban, South Africa. Vitamin C (250 mg/kg) and Glucose strips purchased from Lichro Chemical And Laboratory Supplies were also used.

### Plant

*Hypoxis hemerocallidea* is commonly known as African potato, a genus of the Family Hypoxidaceae (Drewes et al., 1984). The fresh corms of *H. hemerocallidea* were purchased from a local herb-selling shop in Umbilo Road, Durban, KwaZulu-Natal.

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**Extraction of *Hypoxis hemerocallidea***

The corms were authenticated at the Department of Life Science, Westville Campus, University of KwaZulu-Natal, Durban, South Africa. *Hypoxis hemerocallidea* corms were chopped into small pieces and air-dried at room temperature. The dried corms were powdered in a warring blender. Four hundred (400 g) of the powdered corms were soaked in 5 litres of water for 72 hours. The mixture was then filtered, and the filtrate was evaporated at 600 °C using a vacuum rotary evaporator. The wet residue was freeze-dried and stored until ready to use. The powdered extracts obtained contain the bioactive compound hypoxosithe de, sitosterol(Albrecht et al., 1995; Laporta et al., 2007).

**Animals**

Thirty-six (36) adult male Sprague-Dawley rats maintained at the Biomedical Resources Unit (BRU) Animal Facility of the University of KwaZulu-Natal were used. They were supplied with rat feed and water ad libitum, except on days preceding serum assays when they were only allowed access to water 12 hours prior to venipuncture. All the rats were housed in plastic cages (2 rats/cage) having dimensions of 30 x 20 x 13 cm and softwood shavings were employed as bedding in the cages. They were maintained under standardized animal house conditions (temperature: 18-22°C; light: approximately 12 h light per day; humidity: 50-55%) with ethical guidelines adhered to regarding the care and maintenance of the animals as detailed in the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (Health, 1985).

**Grouping of Animals and Dosage of Test Agents/Treatment**

The animals were divided into six (6) groups consisting of 6 rats each. The testing agents were administered daily via oral route with an oral cannula after diabetes was induced using single dose intraperitoneal injection of STZ (65 mg/kg of body weight) in 0.9% NaCl with 100 mM sodium citrate buffer (pH 4.5) (ref) and the treatment lasted for eight (8) weeks. Daily monitoring of the animals including weekly weight and blood glucose was recorded.

Group A = negative controls received distilled water
Group B = diabetic control received citrate buffer
Group C = Diabetic + 50 mg/kg of aqueous extracts of *Hypoxis hemerocallidea* only
Group D = Diabetic + single dose of HAART only
Group E = Diabetic + single dose of HAART and 250 mg/kg of vitamin C
Group F = Diabetic + single dose of HAART+ 50 mg/kg of *hypoxis hemerocallidea*

**Body weight, Blood glucose level, and animals' general well-being**

Daily monitoring of the animal's general well-being was carried out in accordance with the Humane Endpoint score sheet. In addition, the blood glucose level and weight difference were measured and recorded weekly. The whole experiment was conducted over a period of 56 days.

**Biochemical analyses**

Blood samples were analyzed for biochemistry (oxidative stress biomarkers), glutathione [GSH], malondialdehyde [MDA], alanine aminotransferase [ALT], aspartate aminotransferase [AST] (Sher & Hung, 2013).

**Histopathological Studies**

Livers were washed in saline and fixed in 10% neutral buffered formalin for 24 hours. The samples were transferred to a 70% ethanol solution. Ascending grades of alcohol were then used to dehydrate the

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samples and xylene was used as a clearing agent. The samples were immersed in molten paraffin wax at 58°C to 62°C. The prepared blocks were cut into slices of 5 µm) using a microtome (Microtome HM 315, Walldorf, Germany) and stained with hematoxylin and eosin (H&E) stain.

Additionally, the periodic acid-Schiff (PAS) staining technique was used to detect the presence of polysaccharides (e.g., glycogen) and mucosubstances (e.g., glycoproteins, glycolipids, and mucins) in rat liver tissues. The PAS technique is mostly used to evaluate the thickness of the glomerular basement membrane (GBM) in renal disease. The sections were viewed and photographed using an Olympus light microscope (Olympus BX, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co. Ltd., Tokyo, Japan).

**Statistical Analysis**

All results are presented as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Turkey's posthoc test was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla, California, USA), in accordance with the SPSS. Comparisons with $p < 0.05$ were considered statistically significant.

**Ethical Approval**

The study protocol was approved by the University of KwaZulu-Natal Animal Ethics Committee (AREC/010/016PP). The animals received humane care in accordance with the Principle of Laboratory Animal Care of the National Medical Research Council and the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences.

**Results**

***Hypoxis hemerocallidea* effect on total body weight**

Results from Table 1 showed a significant difference ($P<0.05$) in the body weight of the experimental animals. It revealed a significant decrease in body weight of diabetic rats when compared to their initial and final body and with group A (negative control, non-diabetic group). Body weight of rats in groups administered with *H. hemerocallidea* increased steadily from initial to final weight and improved significantly ($p<0.05$) when compared with group B (positive control, diabetic rats without treatment). The rats in group D (HAART and Diabetes) had a significant reduction in body weight when compared with group B (diabetic control).

**Table 1: Body weight difference in experimental animals**

| Groups | BW initial (g) | BW final (g) | BW diff (g) |
|---|---|---|---|
| A | 280 ± 11 | 365.5 ± 19.86 | 85.5 |
| B | 270 ± 22 | 220 ± 19.13* | 50.0 |
| C | 250 ± 13 | 280 ± 35 | 40 |
| D | 280 ±16 | 205 ± 29.43** | 32 |
| E | 270 ± 7.1 | 340 ± 31** | 30 |

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| | | | |
|---|---|---|---|
| F | 254 ± 26.74 | 320 ± 34.94** | 66 |

Data are shown as the mean ± SEM; p<0.05* vs Group A, p<0.05** vs Group B, BW= body weight of rats. Group A: normal control; Group B: diabetic control; Group C: diabetic group fed with 50 mg/kg of *H. hemerocallidea*; Group D: diabetic group fed with HAART; Group E: diabetic group fed with HAART + Vitamin C; Group F: diabetic group fed with HAART and 50 mg/kg of *H. hemerocallidea (H.h).*

***Hypoxis hemerocallidea* effect on serum glucose levels in STZ-induced diabetic rats.**

Results from Table 2 showed a significant difference (p<0.05) in the blood glucose level of the rats. Table 2 showed a significant increase in blood glucose levels of group B (diabetic control) when compared with group A (non-diabetic). The groups administered with 50 mg/Kg H.h had a reduction in the blood glucose levels compared to diabetic control. Notably group E had a significant in blood glucose level compared with diabetic control. There is a significant reduction of AST in groups C and E compared to other groups D and F ( HAART and Diabetic).

**Table 2: Blood glucose and biochemical parameters (ALT and AST) in the experimental groups**

| GROUPS | FINAL BLOOD GLUCOSE (mmol/L) (Mean±SEM) | ALT (U/L) (Mean±SEM) | AST (U/L) (Mean±SEM) |
|---|---|---|---|
| A | 4.7 ± 0.6 | 57 ± 1.6 | 72 ± 7.1 |
| B | 25 ± 3.8* | 94 ± 9.8 | 120 ± 12* |
| C | 22 ± 1.6 | 87.0 ± 4.0 | 52 ± 10** |
| D | 23 ± 1.4 | 88 ± 1.2 | 92 ± 9.0 |
| E | 21 ± 0.97** | 88 ± 0.94 | 58 ± 4.5** |
| F | 22 ± 2.9 | 88 ± 1.2 | 65 ± 9.8** |

Data are shown as the mean ± SEM; p<0.05** vs Group B and p<0.05* vs Group A, AST=alanine amino aspartate, ALT= alanine aminotransferase. Group A: normal control; Group B: diabetic control; Group C: diabetic group fed with 50 mg/kg of *H. hemerocallidea*; Group D: diabetic group fed with HAART; Group E: diabetic group fed with HAART + Vitamin C; Group F: diabetic group fed with HAART and 50 mg/kg of *H. hemerocallidea ( H.h).*

***Hypoxis hemerocallidea* effect on the oxidative stress markers (MDA and GSH).**

The results from Table 3 showed a significant increase in MDA (p<0.05) of rats in diabetic group B (diabetic) when compared to control group A (non-diabetic). MDA level was significantly decreased in group F ( Diabetic + HAART + 50 mg/Kg) compared to diabetic group B. Notably, Group F showed a significant reduction in MDA and an improvement in GSH compared to other treated groups except for Group E ( Diabetic + HAART + Vit C).

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**Table 3: Oxidative stress markers (MDA and GSH) in the experimental groups.**

| Groups | MDA (µm) | GSH (µm) |
|---|---|---|
| **A** | **5.50 ± 3.19** | **8.26 ± 1.75** |
| **B** | **21.19 ± 7.01*** | **5.27 ± 0.24*** |
| **C** | **15.05 ± 16.19*** | **8.25 ± 2.09**** |
| **D** | **16.19 ± 19.95*** | **6.27 ± 0.67** |
| **E** | **5.32 ± 2.86**** | **5.00 ± 0.74*** |
| **F** | **5.70 ± 0.52**/***** | **5.01 ± 0.44*** |

Data are shown as the mean ± SEM; p<0.05* vs Group A, p<0.05** vs Group B, p<0.05*** vs Group C. GSH= reduced glutathione, MDA= malondialdehyde. Group A: normal control; Group B: diabetic control; Group C: diabetic group fed with 50 mg/kg of *H. hemerocallidea*; Group D: diabetic group fed with HAART; Group E: diabetic group fed with HAART + Vitamin C; Group F: diabetic group fed with HAART and 50 mg/kg of *H. hemerocallidea.*

**Histopathology of liver sections**

Figure 1: Hematoxylin and Eosin (H & E) stain showed in group A (non-diabetic) of liver histoarchitecture with central vein within hepatocytes well-arranged. The outlines of hepatocytes and sinusoidal spaces were clearly seen in Fig. 1. Similarly, Figure 6: In (Group F), the liver of diabetic Sprague-Dawley rats treated with HAART and 50mg/kg H. *hemerocallidea* H & E stains showed normal cytoarchitecture of the hepatocyte. Groups C and D showed mild steatosis in the Hepatocyte central vein similar to that of rats in group B (Diabetic). Group E (HAART and 250mg/kg of Vitamin C) showed occluded central vein of occlusion and vacuolization of the hepatocyte.

Periodic acid–Schiff (PAS) stain showed a various degrees of glycogen deposition of liver sections (Fig. 2). PAS, showed slight glycogen deposits in the histoarchitecture of the liver in groups B (diabetic), group C (*50 mg/kg* H. hemerocallidea) and group F (HAART + H. hemerocallidea) showed deeply stained hepatocytes glycogen compared.

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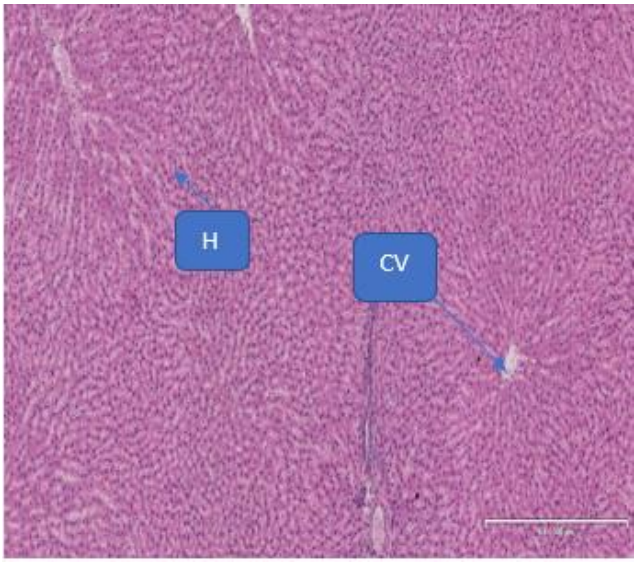
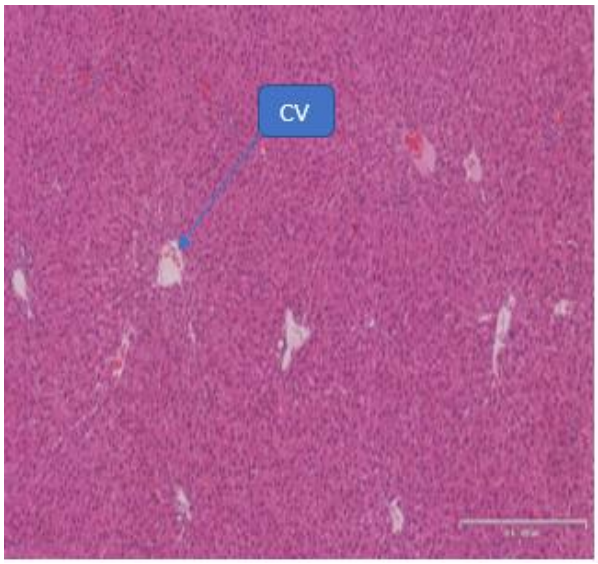
**FIGURES**

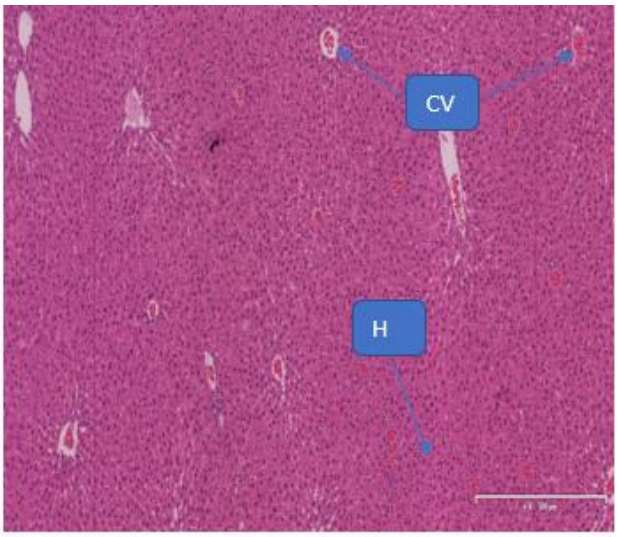
**Figure 1:** Photomicrograph of liver cross sections of Sprague-Dawley rats stained with H&E.



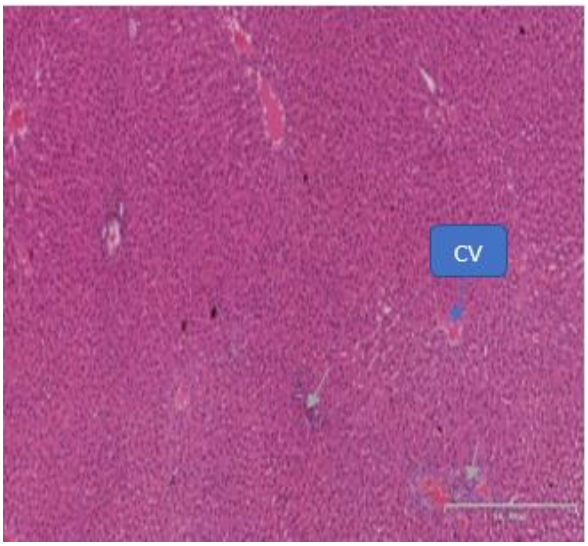

Group A (non-diabetic and untreated). *Note normal Histoarchitecture with hepatocyte and central vein*




Group B (diabetic untreated). *Note: mild steatosis hepatocyte and central vein*




Group C (diabetic and treated with 50 mg/kg H. hemerocallidea only). Note: Normal hepatocyte and central vein with mild steatosis




Group D (Diabetic treated with HAART only) Note: mild steatosis of central vein.

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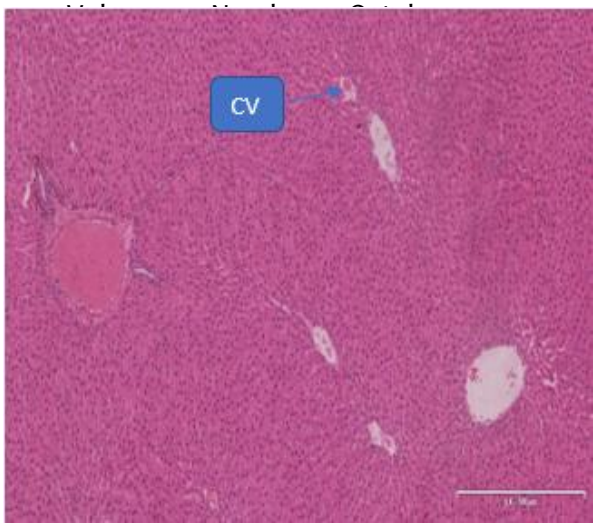
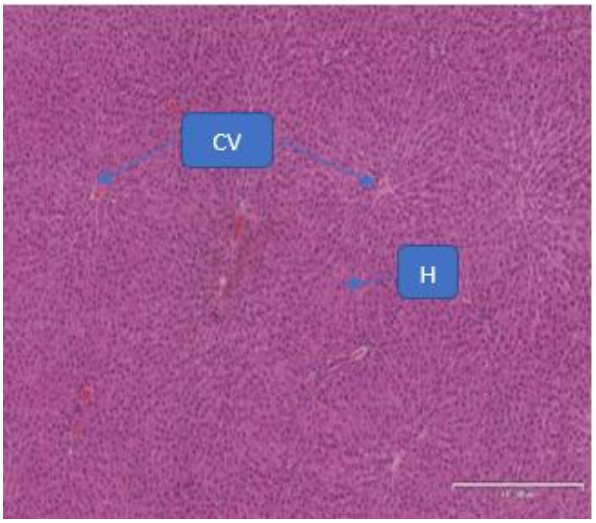



Group E (diabetic and treated with HAART and 250mg/kg Vitamin C). Note: histoarchitecture has occluded central vein and vacuolization of hepatocyte




Group F (Diabetic treated with HAART and 50mg/kg H. hemerocallidea). Note: Normal cytoarchitecture of hepatocyte

Sections labeled (Group A to F). The normal architecture of hepatocellular cords sinusoidal spaces and central vein is observed in control group A and diabetic group F. Note the presence of hepatic steatosis (shown in arrow) in diabetic group B and diabetic groups E and D (mild to severe), accompanied by occluded central vein in groups B, D, and E.
Keys: CV=central vein, H=hepatocytes

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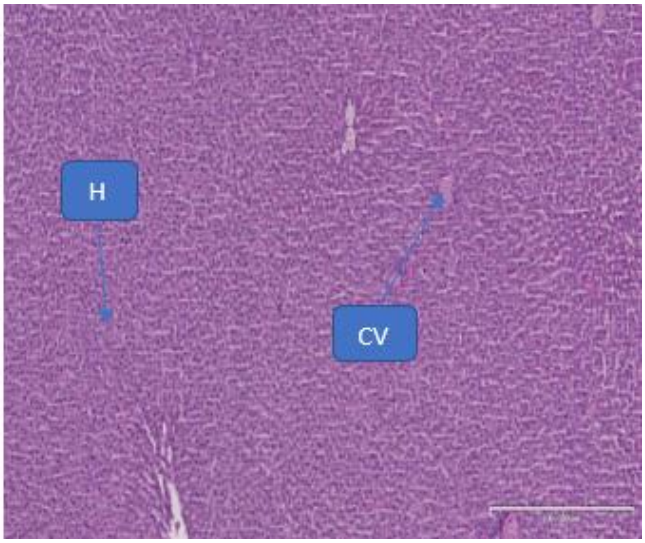
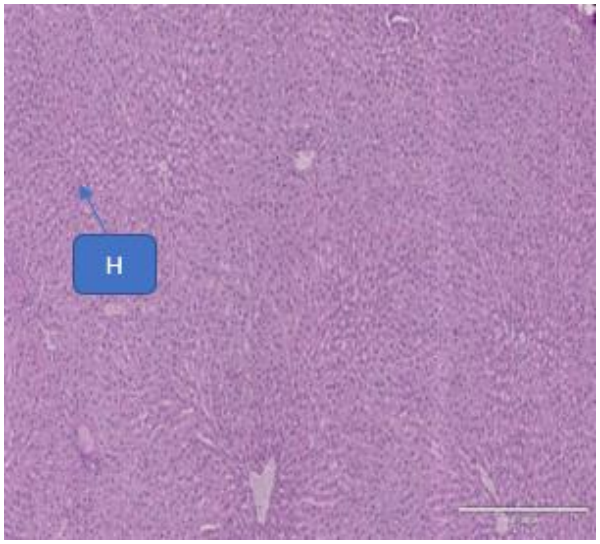
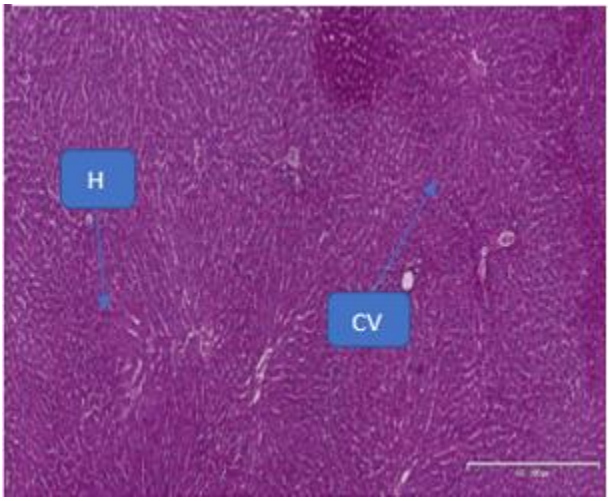
**Figure 2:** Photomicrograph of liver cross sections of Sprague-Dawley rats stained with PAS.



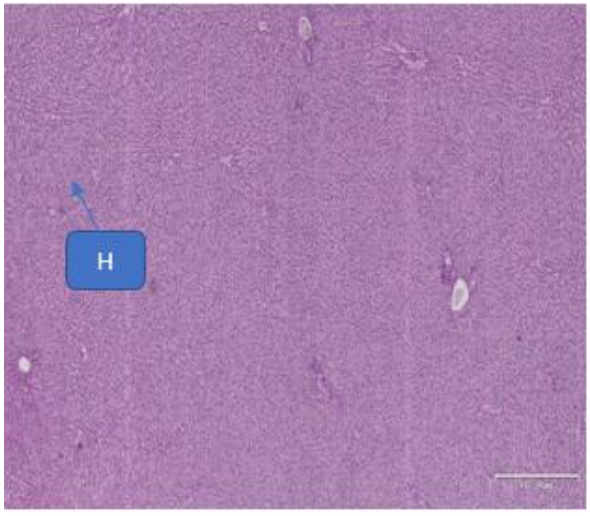

Group A (non-diabetic and untreated). *Note: normal central vein, hepatocyte and sinusoids.*




Group B (diabetic and untreated). *Note: occluded central vein with distorted sinusoids and central vein. Glycogen deposits were*




Group C (treated with 50mg/kg H. hemerocallidea). Note: normal central vein with mild steatosis seen and




Group D *(*Diabetic treated with HAART only*). Note: distorted central veins and devoid glycogen deposits in the histology.*

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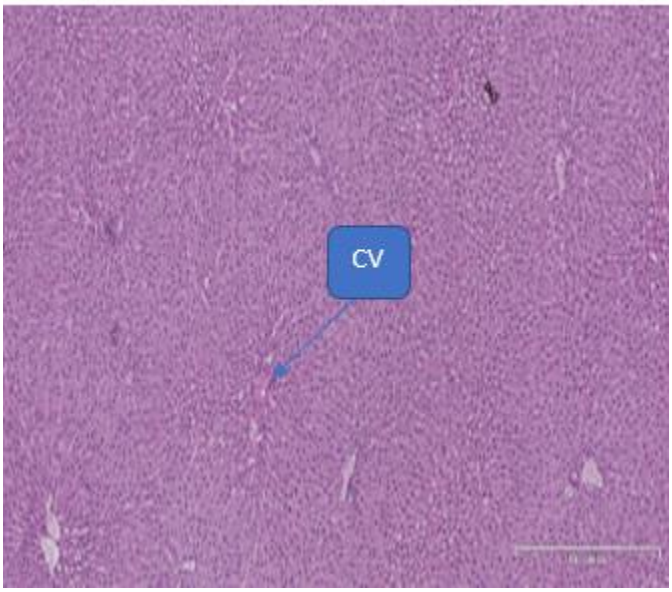
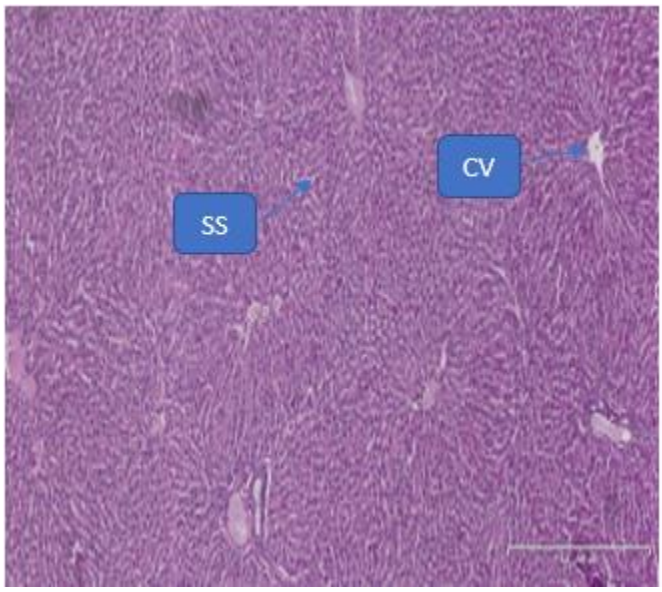



Group E *(*diabetic and treated with HAART and 250mg/kg of Vitamin C*). Note: visible glycogen deposits on the cytoplasm of the cells.*




Group F *(diabetic and* treated with HAART and low dose of 50 mg/kg of *H. hemerocallidea). Note: Normal central vein and normal hepatocytes infiltrated with mild glycogen. between the enlarged sinusoid.*

**Figure 2**: Photomicrographs of liver sections stained with PAS. Normal architecture in control group A and diabetic group F. Note the presence of glycogen deeply stained by PAS in groups A ( non-diabetic) and C (50 mg/kg of *H. hemerocallidea)* compared with Group B, D, and E with poorly stained PAS for glycogen. Keys: CV=central vein, H=hepatocytes, Glycogen

**Discussion**

In many high-income countries, the use of HAART has been associated with a dramatic decline in HIV-associated morbidity and mortality(Pallipamu et al., 2023). Despite this unquestionable success, the prevalence of drug-induced metabolic complications such as DM and insulin resistance has substantially increased (Lawal et al., 2021; Organization, 2007). Moreover, it has been reported that liver diseases are the major complications and leading cause of death in type 2 diabetic people living with HIV(Kalligeros et al., 2023; Verna, 2014). This is so, despite the introduction of various conventional drugs. Due to the high cost of providing treatment and treating its complications, the use of herbal medicine is increasingly encouraged as it has proven to mitigate toxic effects of HAART regimens (Kankara et al., 2022). Medicinal plants possess potent therapeutic metabolites that have been linked to their medicinal values or their activities. The corms of *H. hemerocallidea* are rich in phytosterols (*β*-sitosterol) and hypoxide, and these two compounds have been postulated to be responsible for their antidiabetic and antioxidant effects(Oguntibeju et al., 2016).

Metabolic disorders such as T2DM affect the whole body, causing changes in body weight (Gluckman et al., 2008). In the current study, experimental rats in diabetic control group B and treated group D respectively, showed significant weight loss compared to the negative control group A (non-diabetic). This corresponds to the previous study carried out by Oguntibeju, who mentioned that rapid

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weight loss in DM was due to uncontrolled catabolism of structural proteins as a compensatory response to abnormal carbohydrate metabolism. Furthermore, groups C and F (treated with *H. hemerocallidea*) demonstrated moderate gain throughout the experiment compared to positive control group B (diabetic and untreated) and the rest of the treatment groups: D (treated with HAART only), and E (treated with HAART and Vitamin C). This agrees with the study by Elshawesh (Elshawesh, 2015). who reported that there was no significant gain in body weight of healthy rats and rats treated by *H. hemerocallidea*.

Streptozotocin (STZ) induced DM results in chronic hyperglycemia(Ghasemi & Jeddi, 2023; Lawal et al., 2019). Fasting blood glucose levels within the range of 4.0 to 5.9 mmol/L are considered normal(American Diabetes Association, 2010). Our findings revealed that blood glucose levels remained high in group B (diabetic control) and in treatment groups C, D, E, and F compared to group A. However, treatment groups C, D, E, and F showed lower blood glucose levels compared to the positive control group B. There was a significant blood sugar lowering effect from low dose *H. hemerocallidea*, as also reported by other authors(Bates et al., 2000; Elshawesh, 2015) who found markedly lower fasting glucose in *H. hemerocallidea* treated diabetic rats compared to untreated DM rats.

Diabetes mellitus is associated with several liver abnormalities, such as abnormal glycogen deposition and abnormal elevated hepatic enzymes(Mohamed et al., 2016). The normal value of ALT ranges between 5 to 38 U/L for both males and females. The pathological effects of insulin resistance and hyperglycemia on hepatic tissue are indicated by elevated serum hepatic enzymes(Oguntibeju et al., 2016). To quantify abnormalities in liver function, the serum aminotransferase level was used. In this study, there was no significant difference in ALT level in group C (*H. hemerocallidea*) compared to other groups A, B, D, E, and F ($P<0.05$). The previous study by Saligram(Saligram et al., 2012), reported a high incidence of elevated ALT in patients with newly undiagnosed T2DM, suggesting that the onset of the liver abnormalities is associated with dysglycemia, which may precede diagnosed T2DM. A study by Oguntibeju(Oguntibeju et al., 2016), reported a significant reduction in serum levels of ALT in the diabetic group when treated with *H. hemerocallidea* extract, in contrary to this present study that showed no significant difference.

Aspartate transaminase (AST) found abundantly in the liver is released into the bloodstream following liver injury. Elevated levels of transaminases can reveal hepatic disease or organ damage(Sookoian & Pirola, 2012). It was found that the levels of AST in positive control group B were significantly higher than in negative control group A. However, AST levels were significantly reduced in treatment groups C (treated with *H. hemerocallidea*), E (HAART and Vitamin C), and F ( HAART and H. *hemerocallide*a) compared to control group B (diabetic positive control). The results suggested *H. hemerocallidea* possesses a protective effect on the liver cell. The study by Oguntibeju(Oguntibeju et al., 2016), reported a non-significant reduction in AST in the diabetic group fed with *H. hemerocallidea* when compared to the diabetic control group, which supports our results in this current study.

Oxidative stress has been considered as a conjoint pathological mechanism contributing to the initiation and progression of liver injury. Drug-induced oxidative stress in the liver may result in severe liver diseases, such as Non-alcoholic fatty liver disease (NAFLD)(Li et al., 2015). The present study showed elevated markers of lipid peroxidation (MDA) in diabetic groups B ($P < 0.05$) (untreated diabetic rats) but the reduction in diabetic treatment group C (*H. hemerocallidea*) and with a significant reduction in groups E (HAART and Vitamin C) and F (HAART and *H. hemerocallidea*) compared to normal control group A. Previous study by Vuppalanchi (Vuppalanchi et al., 2011), reported an increase in free radicals leading to the overproduction of MDA. However, Jordaan(Jordaan, 2015), reported a significant decrease when the diabetic group treated with *H. hemerocallidea* was compared with diabetic control, which corroborates with the current study results.

Antioxidant enzymes, such as reduced-GSH, are part of the defense system in the body, which assists in scavenging free radicals(Jordaan, 2015). The results of this study revealed that the levels of GSH

in control group B (untreated diabetic rats) and treatment groups C and D were reduced when compared to control group A. The findings in our study are in line with the previous study that reported a significantly reduced hepatic GSH concentration in diabetic controls when compared with other treatment groups (Oguntibeju et al., 2016). The results of this study revealed that GSH levels, in treatment groups C (diabetic rats treated with *H. hemerocallidea*), showed a significant increase when compared with diabetic group B (Diabetic), group E (treated with HAART AND Vitamin C), and F (treated with HAART and *H. hemerocallidea*). Our findings also revealed non-significant decrease when compared to D (treated with HAART). Our results are supported by the findings of Jordaan(Jordaan, 2015), who also reported a significant increase ($p<0.05$) in GSH levels in *H. hemerocallis-treated* groups when compared to the diabetic group. This result suggests that *H. hemerocallidea* possesses an antioxidant property.

Histoarchitectonic properties of the liver were closely monitored by histological techniques of H&E and special stains. In patients with T2DM, the prevalence of NAFLD is as high as 70%(Ighodaro & Akinloye, 2018; Richard & Lingvay, 2011). Hepatic steatosis is one of the first indicators of liver disease and is common in patients with T2DM(Hazlehurst et al., 2016). H & E liver B, C, D, and E showed mild steatosis while groups F treated with HAART and *H. hemerocallidea)* showed moderate hepatocyte similar to group A(control) (Fig. 1). In line with this study (Julián et al., 2015), reported that there were no histological features of NASH or lobular/portal inflammation present in their study, although moderate steatosis seen in the treated group.

Hyperglycemia is characterized by the presence of glycogen between hepatocytes(Diniz et al., 2020; Soon & Torbenson, 2023). In PAS, glycogen deposits were observed, moderately in the histoarchitecture of the liver in experimental groups C (Fig. 2) when compared with control group A and other treated groups B, D, E and F. Study by Azu(Azu et al., 2016), supported the findings in this study in which the PAS-stained sections revealed the presence of glycogen in the healthy tissues and poorly stained in diabetic tissue.

## Conclusion

This study has demonstrated that the antioxidant and antidiabetic effects of *H.hemerocallidae* mitigated the long-term metabolic effect of antiretroviral therapy in the liver tissue of diabetic Sprague Dawley rats. Still, these protective effects in combination with antiretroviral therapy need further investigation at different doses.

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## Conflict of interest disclosure:

The Authors have no conflict of interest to declare.

## Reference

Albrecht, C., Kruger, P., Smit, B., Freestone, M., Gouws, L., Miller, R., & Van Jaarsveld, P. J. S. A. M. J. (1995). The pharmacokinetic behaviour of hypoxoside taken orally by patients with lung cancer in a phase I trial. *85*(9).

American Diabetes Association. (2010). Standards of medical care in diabetes--2010. *Diabetes Care*, *33 Suppl 1*(Supplement 1), S11-61. https://doi.org/10.2337/dc10-S011

Azu, O. O., Jegede, A. I., Ugochukwu, O., Onanuga, I. O., Kharwa, S., & Naidu, E. C. J. T. R. (2016). Hepatic histomorphological and biochemical changes following highly active antiretroviral therapy in an experimental animal model: Does Hypoxis hemerocallidea exacerbate hepatic injury? , *3*, 114-122.

Bates, S. H., Jones, R. B., & Bailey, C. J. J. B. j. o. p. (2000). Insulin-like effect of pinitol. *130*(8), 1944-1948.

Diniz, A. B., Antunes, M. M., de Souza Lacerda, V. A., Nakagaki, B. N., Lopes, M. A. F., de Castro-Oliveira, H. M., Mattos, M. S., Mafra, K., de Miranda, C. D. M., & de Oliveira Costa, K. M. (2020). Imaging and immunometabolic phenotyping uncover changes in the hepatic immune response in the early phases of NAFLD. *JHEP Reports*, *2*(4), 100117.

Drewes, S. E., Hall, A. J., Learmonth, R. A., & Upfold, U. J. J. P. (1984). Isolation of hypoxoside from Hypoxis rooperi and synthesis of (E)-1, 5-bis (3′, 4′-dimethoxyphenyl) pent-4-en-1-yne. *23*(6), 1313-1316.

Drimie, S. J. A. B. P. p. f. t. F., Agricultural Organization, S.-R. O. f. S., & Eastern Africa. Pretoria, S. A. H. S. R. C. (2002). The impact of HIV/AIDS on rural households and land issues in Southern and Eastern Africa.

Elshawesh, M. A. (2015). *The effects of hypoxis hemerocallidea on blood glucose levels in rats with Type 2 diabetes* University of the Western Cape].

Geronikaki, A., Eleftheriou, P., & Poroikov, V. (2016). Anti-HIV Agents: Current status and recent trends. In *Communicable Diseases of the Developing World* (pp. 37-95). Springer.

Ghasemi, A., & Jeddi, S. (2023). Streptozotocin as a tool for induction of rat models of diabetes: A practical guide. *EXCLI journal*, *22*, 274.

Gluckman, P. D., Hanson, M. A., Beedle, A. S., & Raubenheimer, D. (2008). Fetal and neonatal pathways to obesity. In *Obesity and Metabolism* (Vol. 36, pp. 61-72). Karger Publishers.

Hazlehurst, J. M., Woods, C., Marjot, T., Cobbold, J. F., & Tomlinson, J. W. J. M. (2016). Non-alcoholic fatty liver disease and diabetes. *65*(8), 1096-1108.

Health, N. I. o. (1985). *Guide for the care and use of laboratory animals*. National Academies.

Hovenkamp, E., Demonty, I., Plat, J., Lütjohann, D., Mensink, R. P., & Trautwein, E. A. J. P. i. l. r. (2008). Biological effects of oxidized phytosterols: a review of the current knowledge. *47*(1), 37-49.

Ighodaro, O., & Akinloye, O. J. A. j. o. m. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *54*(4), 287-293.

Ismail, O. O., Isaac, J. A., Ugochukwu, O., Oluwatosin, O. O., Aniekan, P. I., Edwin, N. C., & Onyemaechi, A. O. J. F. h. e. c. (2017). Impaired expression of testicular androgen receptor and collagen fibers in the testis of diabetic rats under HAART: the role of Hypoxis hemerocallidea. *55*(3), 149-158.

Jordaan, A. E. (2015). *The effects of the wild african potato (hypoxis hemerocallidea) supplementation on streptozotocin-induced diabetic wistar rats reproductive function* Cape Peninsula University of Technology].

Julián, M. T., Alonso, N., Ojanguren, I., Pizarro, E., Ballestar, E., & Puig-Domingo, M. J. W. J. o. D. (2015). Hepatic glycogenosis: An underdiagnosed complication of diabetes mellitus? , *6*(2), 321.

Kalligeros, M., Vassilopoulos, A., Shehadeh, F., Vassilopoulos, S., Lazaridou, I., Mylonakis, E., Promrat, K., & Wands, J. R. (2023). Prevalence and characteristics of nonalcoholic fatty liver disease and fibrosis in people living with HIV monoinfection: a systematic review and meta-analysis. *Clinical Gastroenterology and Hepatology*.

Kankara, S. S., Nuhu, A. I., Bindawa, K. A., Haruna, M. R. u., Bello, A., & Abubakar, I. B. (2022). Indigenous traditional knowledge of medicinal plants used for the management of HIV/AIDS opportunistic infections in Katsina State, Nigeria. *Ethnobotany Research and Applications*, *23*, 1-17.

Laporta, O., Pérez-Fons, L., Mallavia, R., Caturla, N., & Micol, V. J. F. C. (2007). Isolation, characterization and antioxidant capacity assessment of the bioactive compounds derived from Hypoxis rooperi corm extract (African potato). *101*(4), 1425-1437.

Lawal, S. K., Adeniji, A. A., Sulaiman, S. O., Akajewole, M. M., Buhari, M. O., & Osinubi, A. A. (2019). Comparative effects of glibenclamide, metformin and insulin on fetal pancreatic histology and maternal blood glucose in pregnant streptozotocin-induced diabetic rats. *Afr Health Sci*, *19*(3), 2491-2504. https://doi.org/10.4314/ahs.v19i3.25

Lawal, S. K., Olojede, S. O., Dare, A., Faborode, O. S., Naidu, E. C. S., Rennie, C. O., & Azu, O. O. (2021). Silver Nanoparticles Conjugate Attenuates Highly Active Antiretroviral Therapy-Induced Hippocampal Nissl Substance and Cognitive Deficits in Diabetic Rats. *J Diabetes Res*, *2021*, 2118538. https://doi.org/10.1155/2021/2118538

Li, S., Tan, H.-Y., Wang, N., Zhang, Z.-J., Lao, L., Wong, C.-W., & Feng, Y. J. I. j. o. m. s. (2015). The role of oxidative stress and antioxidants in liver diseases. *16*(11), 26087-26124.

Matyanga, C. M. J., Morse, G. D., Gundidza, M., & Nhachi, C. F. B. (2020). African potato (Hypoxis hemerocallidea): a systematic review of its chemistry, pharmacology and ethno medicinal properties. *BMC Complement Med Ther*, *20*(1), 182. https://doi.org/10.1186/s12906-020-02956-x

Mills, E., Cooper, C., Seely, D., & Kanfer, I. J. N. j. (2005). African herbal medicines in the treatment of HIV: Hypoxis and Sutherlandia. An overview of evidence and pharmacology. *4*(1), 19.

Mlilo, S., & Sibanda, S. (2022). An ethnobotanical survey of the medicinal plants used in the treatment of cancer in some parts of Matebeleland, Zimbabwe. *South African Journal of Botany*, *146*, 401-408.

Mohamed, J., Nafizah, A. N., Zariyantey, A., & Budin, S. J. S. Q. U. M. J. (2016). Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *16*(2), e132.

Morse, C. G., & Kovacs, J. A. J. J. (2006). Metabolic and skeletal complications of HIV infection: the price of success. *296*(7), 844-854.

Oguntibeju, O. O., Meyer, S., Aboua, Y. G., Goboza, M. J. E.-B. C., & Medicine, A. (2016). Hypoxis hemerocallidea significantly reduced hyperglycaemia and hyperglycaemic-induced oxidative stress in the liver and kidney tissues of streptozotocin-induced diabetic male wistar rats. *2016*.

Ojewole, J. A. J. J. o. E. (2006). Antinociceptive, anti-inflammatory and antidiabetic properties of Hypoxis hemerocallidea Fisch. & CA Mey.(Hypoxidaceae) corm ['African Potato'] aqueous extract in mice and rats. *103*(1), 126-134.

Olarewaju, O. O., Fajinmi, O. O., Naidoo, K. K., Arthur, G. D., & Coopoosamy, R. M. (2022). A review of the medicinal plants with immune-boosting potential. *Journal of Medicinal Plants for Economic Development*, *6*(1), 15.

Olasile, I. O., Jegede, I. A., Ugochukwu, O., Ogedengbe, O. O., Naidu, E. C., Peter, I. A., & Azu, O. O. J. I. j. o. b. m. s. (2018). Histo-morphological and seminal evaluation of testicular parameters in diabetic rats under antiretroviral therapy: interactions with Hypoxis hemerocallidea. *21*(12), 1322.

Organization, W. H. (2007). *WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children*. World Health Organization.

Pallipamu, N., Taheri, S., Thiagaraj, S. S., Shukla, T. S., Gutlapalli, S. D., Farhat, H., Irfan, H., Muthiah, K., & Alfonso, M. (2023). A Systematic Review of How To Reduce Morbidity in HIV Patients With Cardiovascular Diseases. *Cureus*, *15*(2).

Rahman, M. M., Dhar, P. S., Anika, F., Ahmed, L., Islam, M. R., Sultana, N. A., Cavalu, S., Pop, O., & Rauf, A. (2022). Exploring the plant-derived bioactive substances as antidiabetic agent: an extensive review. *Biomedicine & Pharmacotherapy*, *152*, 113217.

Richard, J., & Lingvay, I. J. E. r. o. c. t. (2011). Hepatic steatosis and Type 2 diabetes: current and future treatment considerations. *9*(3), 321-328.

Saligram, S., Williams, E. J., Masding, M. G. J. I. j. o. e., & metabolism. (2012). Raised liver enzymes in newly diagnosed Type 2 diabetes are associated with weight and lipids, but not glycaemic control. *16*(6), 1012.

Sher, Y., & Hung, M. J. B.-p. (2013). Blood AST, ALT and urea/BUN level analysis. *3*(19), e931.

Sookoian, S., & Pirola, C. J. J. W. j. o. g. W. (2012). Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. *18*(29), 3775.

Soon, G. S., & Torbenson, M. (2023). The Liver and Glycogen: In Sickness and in Health. *International journal of molecular sciences*, *24*(7), 6133.

Soriano, V., Puoti, M., Garcia-Gasco, P., Rockstroh, J. K., Benhamou, Y., Barreiro, P., & McGovern, B. J. A. (2008). Antiretroviral drugs and liver injury. *22*(1), 1-13.

Verna, E. C. (2014). Liver biopsy at the time of bariatric surgery: a benefit for patients and the medical community. Seminars in liver disease,

Vuppalanchi, R., Ghabril, M., Chalasani, N., Juluri, R., Bell, L. N., Kamendulis, L., Klaunig, J. E., Saxena, R., Agarwal, D., & Johnson, M. S. J. T. A. j. o. t. m. s. (2011). Oxidative stress in chronic liver disease: relationship between peripheral and hepatic measurements. *342*(4), 314-317.