FMUC FACULDADE DE MEDICINA
UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA- TRABALHO FINAL

KÁTIA SOPHIE SÁ MAURÍCIO

HIV/AIDS: THE MECHANISMS AND CONSEQUENCES OF OXIDATIVE STRESS AND THE BENEFITS OF ANTIOXIDANTS

ARTIGO DE REVISÃO

ÁREA CIENTÍFICA DE DOENÇAS INFECCIOSAS

Trabalho realizado sob a orientação de:

PROFESSOR DOUTOR VÍTOR MANUEL JORGE DUQUE

FEVEREIRO/ 2019

TRABALHO FINAL DO 6º ANO MEDICO

HIV/AIDS: THE MECHANISMS AND CONSEQUENCES OF OXIDATIVE STRESS AND THE BENEFITS OF ANTIOXIDANTS

Aluna: Kátia Sophie Sá Maurício

Afiliação: Faculdade de Medicina, Universidade de Coimbra, Portugal

Endereço de Correio Eletrónico: kmauricio13@gmail.com

Orientador: Professor Doutor Vítor Manuel Jorge Duque

Afiliação: Faculdade de Medicina, Universidade de Coimbra, Portugal

Endereço de Correio Eletrónico: Duque.vitor@gmail.com

TABLE OF CONTENTS

ABSTRACT

Studies have shown that human immunodeficiency virus-1 (HIV-1) infection generates elevated levels of reactive oxygen species (ROS) and that the ineffective neutralisation of these species promotes oxidative stress (OS). This in turn contributes to the depletion of important cells within the immune system, the pathogenesis of co-morbidities unrelated to acquired immunodeficiency syndrome (AIDS) and ultimately, the development of AIDS. In addition, data suggests that patients present with impaired antioxidant defence systems. The administration of antioxidant supplementation could benefit them greatly.

In this review we explore the various mechanisms that viral proteins Tat, Vpr and gp120 utilise to trigger massive ROS production and how this can lead to disturbances in the immune system (CD4+ T-cell depletion and induction of latency in macrophages), central nervous system (CNS) toxicity and pathology of the cardiovascular (CVS) and respiratory system. The implication that highly active antiretroviral therapy (HAART) potentiates the oxidative effects of HIV-1 is briefly explored.

Finally, we conducted a thorough study of the literature to determine whether antioxidants such as N-acetylcysteine (NAC), vitamin A, C and E, and polyphenols, have any significant impact on ROS levels, disease progression and the reduction of comorbidities. Hence, helping to determine whether they can play a role in the treatment of HIV/AIDS with the purpose of increasing the quality of life of these individuals.

KEYWORDS

HIV-1, Reactive Oxygen Species, Oxidative stress, Viral Proteins, HAART, Antioxidants

ABREVIATIONS

$^1O_2$- Singlet oxygen

4-HNE- 4-Hydroxynonenal

8-OxoG- 8-oxoguanine

AGE- Advanced glycation end products

AIDS- Acquired immunodeficiency syndrome

ANT- Adenine nucleotide translocase

AOPP- Advanced oxidation protein products

ARE- Antioxidant response elements

ART- Anti-retroviral therapy

BBB- Blood brain barrier

CAT- Catalase

CNS- Central nervous system

CRI- Co-receptor inhibitors

CVD- Cardiovascular disease

DNA pol-γ- DNA polymerase gamma

EPR- Electron paramagnetic resonance

Fe^{2+}- Iron

FI- Fusion inhibitors

Gp120- Glycoprotein 120

GPXs -Glutathione peroxidase

GR- Glutathione reductase

GSH- Glutathione

GST- Glutathione S-transferase

H_2O- Water

H_2O_2- Hydrogen peroxide

HAART- Highly active antiretroviral therapy

HAND- HIV-associated neurocognitive disorder

HAT- Histone acetyltransferase

HIV-1- Human immunodeficiency virus 1

ICAM-1- Intercellular Adhesion Molecule 1

INI- Integrase inhibitors

LTR- Long terminal repeat

M-CSF- Macrophage colony stimulating factor

MCP-1- Monocyte chemoattractant protein-1

MDA-Malondialdehyde

MMPs- Matrix metalloproteinases

MTCT- Mother-to-child transmission

NADPH- Nicotinamide adenine dinucleotide phosphate

NF-κB - Nuclear factor kappa-light-chain-enhancer of activated B-cells

NNRTI- Non-nucleoside reverse transcriptase inhibitors

NRTI- Nucleoside reverse transcriptase inhibitors

NtRTI- Nucleotide reverse transcriptase inhibitors

$O_2^{\bullet-}$- Superoxide

OH·- Hydroxyl radical

OS- Oxidative Stress

OSI- Oxidative stress index

oxLDL- Oxidised low-density lipoprotein

PCC- Protein carbonyl content

PI- Protease inhibitors

PLWH- People living with HIV-1

PRR- Pathogen recognition receptor.

PRXs- Peroxiredoxins

PUFA- Polyunsaturated fatty acids

ROS- Reactive oxygen species

SOD- Superoxide dismutase

TAP- Total antioxidant potential

TAR- Transactivation response

TAS- Total antioxidant status

TAT- Trans-activator of transcription

TPP- Total plasma peroxide

VCAM-1- Vascular cell adhesion protein 1

Vpr- Viral protein r

INTRODUCTION

The human immunodeficiency virus (HIV-1) is a Retrovirus of the Lentivirus genus that primarily infects cells of the immune system and with time leads to acquired immunodeficiency syndrome (AIDS). There are currently around 36.9 million people living with HIV-1 (PLWH) worldwide and 21.7 million with access to anti-retroviral therapy (ART) [1]. HIV-1 was once a life-sentence with death by AIDS occurring within 9-11 years of diagnosis, but since the introduction of ART the average life expectancy has greatly increased [2]. This means that people are now living longer with this disease, around 13% of the adult population living with HIV-1 are aged 50 and over [3]. Despite effective viral control, PLWH experience excess morbidity and mortality compared to the general population. Age-related illnesses such as cardiovascular disease, cognitive impairment, malignancies and osteoporosis seem to be more prevalent in these individuals, but with a tendency to arise at a much younger age than the general population [4]. As a result of this it has become extremely important to try and discover why PLWH are more susceptible to these diseases and if there are ways to halt disease progression.

The association between oxidative stress (OS) and HIV-1 pathogenesis and its progression to AIDS has been around for many years now [5]. Oxidative stress has been implicated in many aspects of HIV-1 pathogenesis such as increased HIV-1 replication, impairment of CD4+ T-cells, altered immune response and antiretroviral drug toxicity [6]. The causative agents of OS are known as reactive oxygen species (ROS). ROS are generated and play crucial roles in many physiological processes within cells, but when they are produced in elevated levels and not neutralised by defence mechanisms they are extremely destructive, damaging cellular macromolecules such as DNA, lipids and proteins which in turn leads to disease [7]. Studies have shown that HIV-1 patients have alterations in their antioxidant defence mechanisms and due to this are more likely to suffer excessive OS [8]. Several studies have shown that antioxidants are a promising, natural and inexpensive remedy capable of altering HIV-1 progression to AIDS [9]. Since around 70% of PLWH live in sub-Saharan Africa [1], where access to ART is not always financially possible means that finding an inexpensive method to slow disease progression and help combat its co-morbidities is essential.

The role of ROS in HIV-1 infection is complex, making it necessary comprehend the mechanisms that HIV-1 utilises to generate OS and the ways in which the pathological process can be halted. The present review aims to explore the mechanism through which HIV-1 is capable of generating OS and the consequences of these processes. We shall briefly explore how ART can also be to blame for this state of excessive ROS production. Lastly, we review how effective antioxidants such as N-acetylcysteine (NAC), vitamin A, C and E, and polyphenols are in the modification of these pathological processes.

METHODS

The literature review was conducted using PubMed and Google Scholar databases. Searched items included keywords: “HIV-1 or Human deficiency virus- 1”, “AIDS or acquired immunodeficiency syndrome”, “HIV/AIDS” combined with two of the following “ROS or reactive oxygen species”, “OS or oxidative stress”, “viral proteins”, “antioxidants”, “polyphenols”, “HAART or highly active antiretroviral therapy”, “co-morbidities”. Eligibility criteria included English language, articles published in peer-reviewed journals with preference given to articles published in the last 10 years. However, articles containing clinically significant data that were published before this were not excluded. Other sources were also searched including UNAIDS and National Institute of Health- Office of Dietary Supplements (accessed online January 2019). Studies were selected for inclusion based on eligibility criteria above and relevance to topics discussed.

SUMMARY OF EVIDENCE

1. REACTIVE OXYGEN SPECIES, OXIDATIVE STRESS AND ANTIOXIDANTS

ROS are small short-lived oxygen-containing molecules that are chemically highly reactive due to an unpaired electron and are produced as by-products of cellular metabolism in aerobic cells. Examples include, superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH·) and singlet oxygen ($^1O_2$) [10]. Superoxide anions and hydrogen peroxide are the most common ROS within the body, these are generated by different mechanisms in different intracellular locations. There are 3 main sources of superoxide anions within the cells. The first and largest source is the mitochondria during oxidative phosphorylation; complexes I and III from the respiratory chain proteins "leak" electrons and these rapidly react with mitochondrial O_2 leading to the formation of superoxide ($O_2^{\bullet-}$) (**FIG. 1**) [11,12]. The second is by a family of NADPH oxidases, there are seven isoforms of these enzymes: NOX1-NOX5 and DUOX1/DUOX2 [13]. The third is through cytochrome P450, an enzyme responsible for the metabolism of potentially toxic substances such as drugs and many endogenous compounds [14]. Hydrogen peroxide is mainly generated as a by-product of catabolic reactions and when disulphide bonds are formed during protein folding in the lumen of the endoplasmic reticulum (ER). Other sources of ROS include degradation of lipids and amino acids [15].

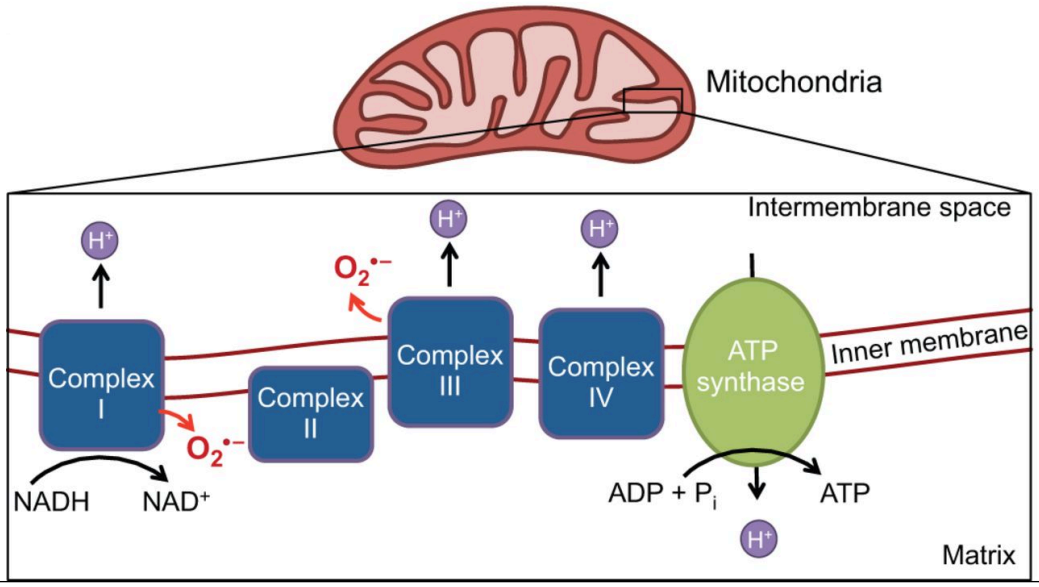

FIGURE 1: PRODUCTION OF ROS IN THE MITOCHONDRIA DURING OXIDATIVE PHOSPHORYLATION.
(Adapted from [12])

Not all ROS have the same degree of reactivity with other biological molecules. The most reactive is the hydroxyl radical, its frantic search for stability sees this radical reacting with nearly any molecule in its vicinity such as with lipids in cell membranes, nucleotides in DNA and sulfhydryl groups in proteins, among many others, all this resulting in extensive tissue damage. The second most reactive

is the superoxide anion and the most stable and least reactive is hydrogen peroxide. Hydrogen peroxide has the capacity to diffuse small distances and cross membranes making it a very important second messenger [16]. Its downfall is that it can be converted into various molecules, with the main one being the hydroxyl radical, as mentioned above this radical has a much higher oxidising capacity and can cause irreversible damage to nearby molecules.

Eukaryotic cells have developed specialised antioxidant systems in order to neutralise ROS and protect themselves from the extensive damage caused by these entities. Antioxidants are naturally occurring or synthetic compounds that have the ability to inhibit oxidation, thus preventing the formation of free radicals and the damage they induce by scavenging free radicals and promoting their decomposition within the body. The neutralising capabilities of these antioxidants result from the fact that they can donate an electron, therefore stabilising the highly unstable free radicals or that they can convert ROS into other less destructive molecules (14).

Within the body exists 2 groups of antioxidants, the majority are generated endogenously, and the rest are obtained from a balanced diet. The first are small, non-enzymatic molecules such as glutathione (GSH), pyruvate, vitamin C and vitamin E that directly neutralise the free radicals. The second are antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxiredoxins (PRXs), glutathione peroxidases (GPXs) and glutathione reductase (GR). SODs are responsible for the conversion of the superoxide radical into hydrogen peroxide. The neutralisation of hydrogen peroxide can be achieved by CAT, GPx and PRXs (14). These enzymes have different isoforms that are situated in different locations within the cell, SOD1 is mainly found in the cytoplasm, SOD2 in the mitochondrial matrix and SOD3 on the cell surface. This means that the biological molecules that they protect are different, for example GPX4 protects the cell membrane from oxidative damage by neutralising lipid peroxides [17].

It is important to mention that the expression of a vast number of antioxidant enzymes are under the control of the transcription factor Nrf2, the main mediator of cellular adaptation to redox stress. In states of equilibrium Nrf2 is found in the cytoplasm where it is constantly degraded by its inhibitor, INrf2. When the cell begins to enter an oxidative state, certain tyrosine kinases are activated, and these then phosphorylate Nrf2, this prevents its degradation by INrf2. Nrf2 is now free to enter the nucleus where it attaches to antioxidant response elements (ARE) thus promoting the transcription of these genes [18].

There are several techniques that can be utilised to determine the cell's redox state and measure ROS levels. This information has been summarised in **figure 2** [19].

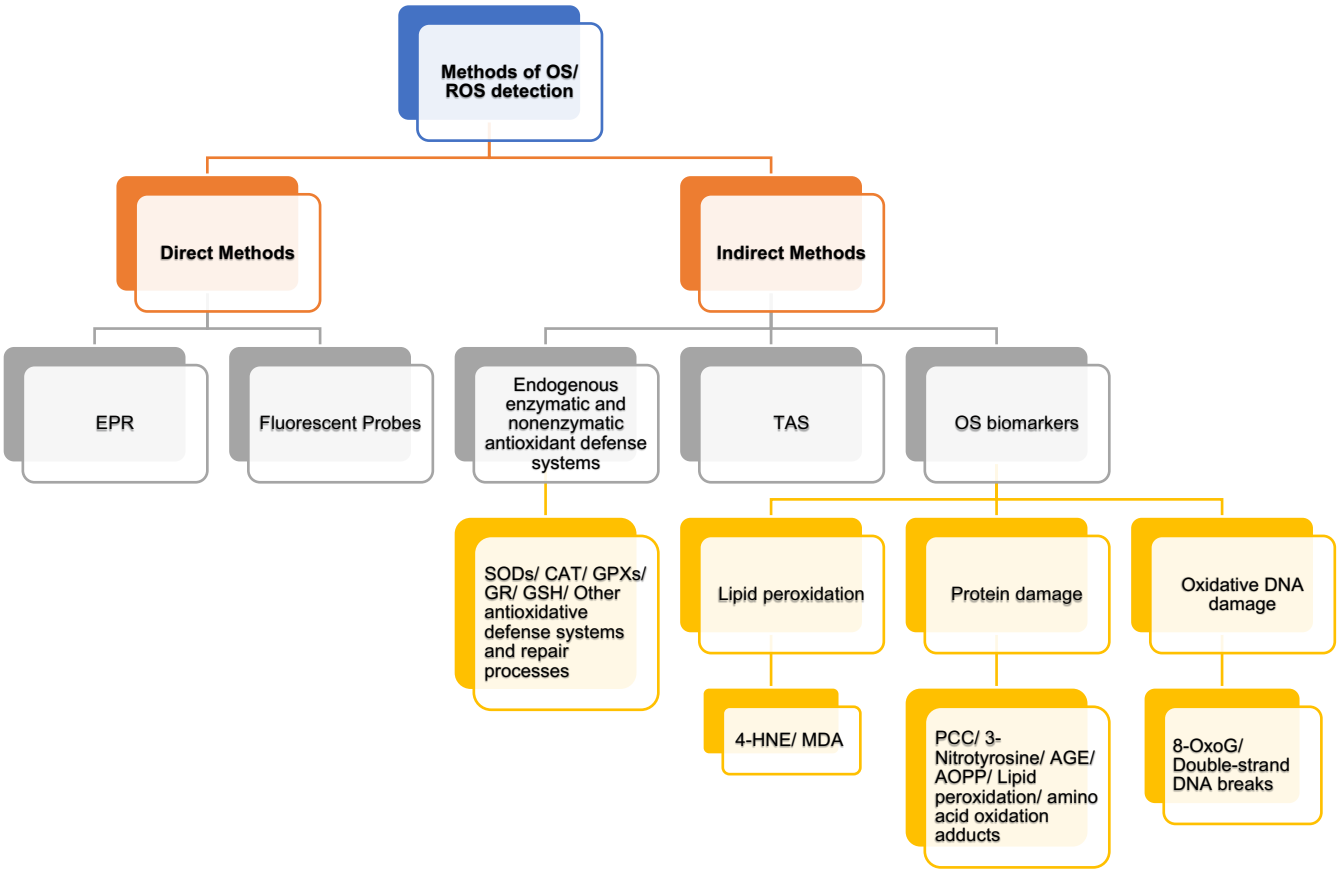

FIGURE 2: METHODS OF MEASURING OXIDATIVE STRESS. There are 2 main ways in which levels of ROS can be detected within cells. The direct methods, as the name indicates, directly measure the cellular levels of ROS- EPR detects oxygen radicals; fluorescent probes consists of the use of low molecular weight compounds (sensors) that can be oxidised by ROS generating fluorophores, the level of fluorescence produced can be measured. The indirect methods quantifies the levels of compounds associated with increased ROS production, such as antioxidants utilised in neutralisation, by products of cell damage and TAS. 4-HNE = 4-Hydroxynonenal; 8-OxoG= 8-oxoguanine; AGE = advanced glycation end products; AOPP = advanced oxidation protein products; CAT= catalase; EPR= Electron paramagnetic resonance; GPXs= glutathione peroxidase; GR= glutathione reductase; MDA = malondialdehyde; PCC = protein carbonyl content; SODs= superoxide dismutase; TAS= total antioxidant status. (Adapted from [19])

In summary, under normal physiological conditions, tissue damage is kept to a minimum thanks to the action of both enzymatic and non-enzymatic antioxidants that keep ROS concentrations at a necessary low. At a low concentration, ROS act as signalling molecules where they partake and help regulate various physiological processes such as relaying information from membrane receptors, protecting cells from infectious pathogens, and most importantly maintaining a redox homeostasis. Dysregulation of this carefully controlled system leads to oxidative stress. Oxidative stress ensues when there is an imbalance between ROS production and the body's ability to efficiently sequester the reactive intermediates or to repair the resulting damage. A major consequence of OS is that it can

severely compromise cell viability due to the extensive damage exacted on cellular components such as nucleic acid, proteins and lipids, this in turn leads to further generation of secondary ROS as well as the activation of various cellular responses that result in cell death, either by apoptosis or necrosis (**FIG. 3**) [12].

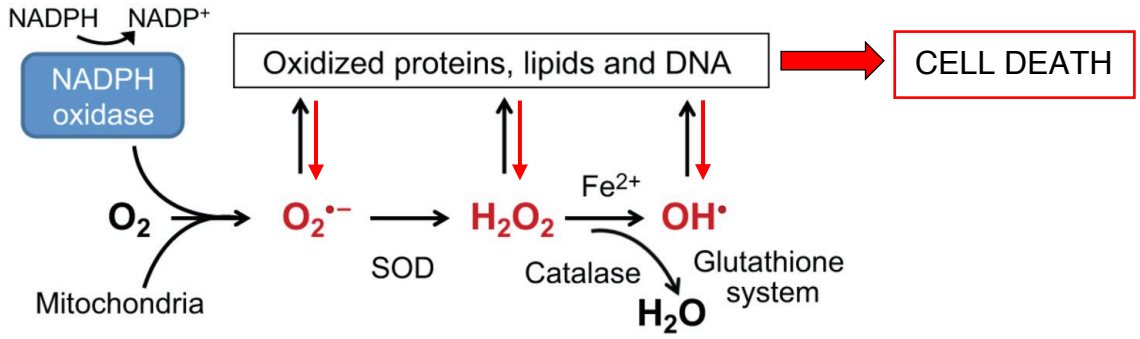

FIGURE 3: ROS GENERATION. Superoxide ($O_2^{\bullet-}$) can be reduced to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). Catalase can then further reduce H_2O_2 into water (H_2O) or it can spontaneously oxidise iron (Fe^{2+}) to form the highly reactive hydroxyl radical (OH·). When ROS production exceeds the body's antioxidant capacity, damage to cellular components occurs (**black arrows**). This leads to further ROS production (**red arrows**). When damage is too great to repair, cell death is induced. (Adapted from [12])

2. OXIDATIVE ROLE OF HIV-1

The first observation of OS in HIV-1 pathology occurred a few years after the discovery of HIV-1. To date, an abundant amount of evidence exists, both in vitro and in vivo, that demonstrate that HIV-1 infection triggers an increase in OS within the body. ROS production induced by HIV-1 is mediated by certain proteins located on the viral envelope such as trans-activator of transcription (Tat), viral protein r (Vpr) and glycoprotein 120 (gp120). Throughout this section we shall explore the various mechanisms used by HIV-1 to promote OS and also some of the consequences that result from this.

2.1 MECHANISMS OF ROS PRODUCTION

2.1.1 TAT

Tat is an essential HIV-1 regulatory protein with a multitude of effects on various cellular and viral functions. Its main role is to promote high levels of viral gene expression through its interaction with HIV-1 transactivation response (TAR) element RNA. It also regulates at least two of the steps in HIV-1 m-RNA processing [20]. Expression of a mutant form of Tat known as Null-basic in HIV-1 infected cells resulted in inhibition of HIV-1 production and infectivity. Null-basic strongly inhibited HIV-1 reverse transcription meaning that HIV-1 replication was not possible in those cells, this resulted in undetectable levels of cell-to-cell HIV-1 transmission. It was also shown that human T-cell lines and primary human CD4+ T-cells that constitutively expressed Null-basic were protected from HIV-1 infection, indicating that Null-basic is a potent HIV-1 inhibitor [21,22].

Extracellular and intracellular Tat frequently have opposite actions. Intracellular Tat can delay apoptosis of HIV-1 infected cells and when it is released and taken up by adjacent non-infected cells it can be a potent inducer of cell death. Rodríguez-Mora et al. used Tat 101Jurkat cells as models of non-infected cells that had taken up Tat. The results showed that intracellular Tat dysregulated the expression of 19 cellular proteins related to metabolism and control of oxidative stress, those related to OS are SH3 domain-binding glutamic acid-rich-like protein 3 (SH3BGR3), Peroxiredoxin-1 (PRDX1), protein disulphide-isomerase (P4HB), thioredoxin domain-containing protein 17 (TXDC17), Superoxide dismutase (SOD1), protein disulphide-isomerase A4 (PDIA4), Tropomyosin alpha-4 (TPM4), Phosphatidylethanolamine-binding protein (PEBP1) and protein disulphide-isomerase A3 (PDIA3). The expression of genes related to mitochondrial membrane stabilization were also affected, this led to decreased ATP production mainly because of decreased complex I activity. This promoted an increase in the number of mitochondria but not sufficient to overcome this impairment. The mitochondria are one of the main sources of ROS and in Jurkat Tat101 cells the mitochondria are compromised, making it more likely that ROS are produced in greater amounts and this was evident by the data. The levels of ROS were significantly higher in Jurkat Tat101 cells when compared to control. It was also shown that Tat promoted apoptosis in these cells [23].

HIV-1 positive individuals are more at risk of developing certain types of cancers. B-cell lymphomas are the most common types of HIV-related cancer and their development has been linked to the Tat protein secreted by HIV-1 infected cells. HIV-1 itself is unable to infect B-cells but Tat can penetrate many cells that HIV-1 cannot. El-Amine et al. showed that incubation of B-cells isolated from

healthy donors with purified Tat protein led to induction of mitochondrial OS, glutathione (GSH) deficiency, DNA damage and appearance of chromosomal aberrations, all of which can contribute to oncogenic transformation. In blood samples from HIV-positive subjects, B-cells had significantly higher levels of OS and DNA damage when compared to healthy controls [24].

2.1.2 VPR

Viral protein R (Vpr) is an accessory HIV-1 protein encoded by the Vpr-late gene with the capability to cause structural and functional damage in many types of eukaryotic cells through the induction of redox imbalance. Vpr can either be found inside or outside host cells. Inside the host cells it is mainly found in the nucleus, cytoplasm and mitochondria. Outside of the host cells, Vpr can be free or packaged into virions in the plasma and cerebrospinal fluid. Vpr increases viral replication of T cells, it promotes HIV-1 infection of macrophages and is responsible for disease progression. It does this by arresting infected cells in G2 of the cell cycle and inducing a mitochondria-dependent apoptotic pathway. In cells that were injected with Vpr, a vast amount of damage to membranes was observed. Vpr binds to adenine nucleotide translocase (ANT) in the inner mitochondrial membrane to induce formation of a pore and increase mitochondrial membrane permeability which causes mitochondrial dysfunction [25]. Due to this there is a decrease in ATP production and this has been linked to a decrease in GSH production, helping to promote an oxidative state within the cells. The levels of Vpr are higher in patients with late stage disease than those that are asymptomatic or in the latency phase. In cells that were treated with recombinant Vpr, a significant accumulation of ROS was observed [26]. It is known that ROS provoke extensive cellular damage and is involved in the pathogenesis in both AIDS and non-AIDS diseases.

2.1.3 GP120

Gp120 is an envelope glycoprotein located on the outer surface of the HIV-1 envelope. This protein is essential for the initial binding of HIV-1 to it target cells and facilitates viral entry. In CD4+ T-cells, gp120 binds to the CD4, this interaction induces a conformational change that allows fusion of viral membrane with that of the host. Gp120 has been shown to play a very important role in the crossing of the blood brain barrier (BBB) and the infection of the various cells located in the brain. It has been shown that brain endothelial cells exposed to gp120 display reduced levels of intracellular GSH, GPx, and GR and increased levels of MDA, showing that the cells were oxidatively challenged. There was also a decrease in the ratio of GSH/GSSG, a widely accepted indicator of oxidative stress [27,28]. Similar results were obtained in investigations using kidney tubular cells. Gp120-transduced cells had increased expression of the death receptor Fas and its ligand, FasL and a rise in caspase-8 activation leading to the induction of programmed cells death. Gp120-transduced cells displayed greater rates of apoptosis when compared to control. This effect of gp120 could be inhibited both by anti-FasL antibody and caspase-8 inhibitor [29]. Gp120 also appears to mediate some of its effects through the activation of the NF-κB pathway [30].

2.1.4 NF-κB

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a transcription factor that controls and regulates expression of a multitude of genes involved in stress responses, inflammation and programmed cell death. It can either induce pro- or antioxidant pathways depending on the cell's redox state. In the cytoplasm, NF-κB is bound and kept inactive by inhibitory factor, IκB. Exposure of cells to extracellular stimuli that perturb redox balance results in rapid phosphorylation, ubiquitination, and proteolytic degradation of IκB, NF-κB is liberated and free to translocate into the nucleus where it regulates gene transcription. NF-κB is involved in various signalling pathways that can be activated by pro-inflammatory cytokines such as tumour necrosis factor-α (TNF- α) and interleukin-1 (IL-1). TNF- α activates protein kinase cascades that lead to the phosphorylation of IκB and subsequent activation of NF-κB. It can also be activated by ROS such as hydrogen peroxide. Most of the pathways activated then lead to apoptosis [31]. The effects of Tat are mediated by NF-κB activation; Tat sequesters IκB thus allowing NF-κB to translocate into the nucleus, where it acts as a prooxidant. When IκB is inhibited in Tat-treated B-cells there is a significative decrease in ROS production and DNA damage (23).

Latency occurs when HIV-1 remains in a dormant state within certain immune cells, when these cells are activated the virus begins to replicate again. Low levels of NF-κB appears to have an important role in the establishment of HIV-1 latency in resting $CD4^+$ T-cells. The HIV-1 long terminal repeat (LTR) presents with binding sites for NF-κB, when NF-κB binds to it histone acetyltransferase (HAT) is recruited and acetylates the histone tails, which opens up the nucleosomes to facilitate HIV-1 transcription. Various HIV-1 subtypes contain different numbers of NF-κB binding sites. Subtype E contains one, subtype B has two and subtype C can have three or four. These variations could mean that certain subtypes are more likely to be reactivated by NF-κB than others. In order for NF-κB to be free its inhibitory factor needs to be inactivated. There are 6 known human IκB proteins; IκB is mostly expressed in T-cells and its degradation plays an important role in the reactivation of HIV-1 from latency [32,33].

2.2 CONSEQUENCES OF ROS PRODUCTION

2.2.1 CD4+ T-CELL DEPLETION

Immunodeficiency as a result of CD4+ T-cells depletion is a hallmark of HIV-1 infection. Apoptosis is thought to be one of the main mechanisms responsible for the evident CD4+ T-cell loss that is observed during HIV-1 infection and that contributes to disease progression to AIDS [34]. It was once thought that the only CD4+ T-cells that died were those that were infected by HIV-1, but it seems that non-infected, bystander CD4+ T-cells located in the lymph nodes are also targeted and that these account for 95% of the cells lost. The exact mechanisms behind this loss is not entirely known, some believe that the following factors are at play, TNF- α, FasL, TRAIL, and various viral factors (e.g. Tat, Vpr, gp120) released from infected cells [35]. Excessive induction of apoptosis greatly compromises the immune system, thus lowering the body's resistance to the virus. Data exists that shows that HIV-

1 induced apoptosis is preceded by an exponential increase in ROS generation by the mitochondria in Jurkat-tat cells, showing that Tat seems to play an important role in this process. A progressive depletion of intracellular GSH levels was also evident [36].

FAS-mediated cell death may play an important role in induced immune damage and enhanced disease progression. A study conducted in elderly HIV-1 infected individuals examining the relationship between CD4+ T-cell count, apoptosis and FAS expression showed these individuals displayed high levels of apoptosis which correlate with levels of FAS expression. The more FAS that is expressed by the CD4+ T-cells, the more likely the cell is to undergo programmed cell death. It was also shown that there is a difference in the phenotype and properties of CD4+ T-cells between young and elderly individuals. It seems that older individuals have a higher percentage of memory T-cells (HIV-1's primary target) and a lower number of naïve CD4+ T-cells (relatively resistant to HIV-1 infection). To add to this, cells also showed less CD4 expressed on their surface; reduced levels of CD4 seem to facilitate efficient virus release and enhance its infectivity. The combination of all these factors, increased numbers of CD4+ T-cells that are highly susceptible to HIV-1 infection as well as to FAS-mediated cell death could explain why older individuals experience a greater loss of CD4+ T-cells and a faster progression to AIDS compared to their younger counterparts [37].

2.2.2 LATENCY IN MACROPHAGES

Macrophages are a type of white blood cells derived from monocytes. The main role of these cells is to engulf and digest cellular debris, eliminate pathogens from the body and to stimulate lymphocytes. They play a fundamental role in initial infection and contribute to HIV-1 pathogenesis throughout the course of viral infection. They are among the first cells to be infected by the virus and their long-life span makes them important viral reservoirs. These cells, unlike CD4+ T-cells that die within a few days of HIV-1 infection, are capable of surviving for extended periods of time, several weeks to years. It has been shown that cells of the monocyte-macrophage lineage are extremely resistant to viral cytopathic effects and apoptosis and exhibit longer life spans even when they are exposed to different oxidative stress stimuli [38]. As mentioned previously, HIV-1 infection leads to OS. Viral infection of macrophages provides better protection against OS which could be an important viral strategy to make HIV-1-infected macrophages long lived and more resistant viral reservoirs. HIV-1 infected macrophages express macrophage colony stimulating factor (M-CSF); this cytokine upregulates the anti-apoptotic genes such as Bfl-1 and Mcl-1 and down-regulates TNF-related apoptosis inducing ligand (TRAIL-R1/DR4), this results in macrophages resistant to apoptosis [39]. In addition, HIV-1 infection of macrophages facilitates transmission and establishment of HIV-1 infection in resting CD4+ T-cells and play an important role in their loss (26). The viral proteins that modulate the action of infected and bystander macrophages to facilitate disease progression are Nef, Tat, Vpr, and gp120 [40–42].

In summary, there are quite a few reasons that explain why macrophages are so important in the pathogenesis of HIV. These cells provide the perfect environment for the production of viral reservoirs since they are long-lived, widely distributed throughout the body and resistant to apoptosis.

HIV-1 is able to control macrophage survival through the upregulation of various antiapoptotic genes, an example being the increased expression of transcription factors such as NF-κB that has the ability to prevent TNF-induced apoptosis. HIV-1 can manipulate the macrophages to secrete chemokines thus recruiting CD4+ T-cells in order to facilitate virus transfer to these cells and subsequently their death. Furthermore, activated macrophages lead to the depletion of CD4+ T-cells, resulting in immune deficiency. All these actions help accelerate disease progression attributable to ineffective clearance of apoptotic cells, establishment of chronic inflammatory state and diminution of vital immune cells. These cells are also more resistant to ART. Therefore in order to effectively control disease, therapies have to be able to eliminate these reservoirs and it seems that antioxidants could be the way forward [43].

2.2.3 CNS TOXICITY

Despite living in the era of combined ART, HIV-associated neurocognitive disorder (HAND) continues to be prevalent. Less severe forms of HAND are now more common, with the more severe forms, HIV-associated dementia, being quite rare. Even so, this disorder continues to be important as it has a great impact on survival, quality of life and everyday functioning [44].

There are two main pathways that HIV-1 can use to bring about neuronal damage, the direct and indirect pathway [45]. The **direct pathway** entails the crossing of the blood-brain barrier (BBB) in order to infect the CNS, there are two main ways that this can be achieved:

1) The "trojan horse" hypothesis **(FIG. 4: 1a, 1c)**: Outside of the CNS, HIV-1 infects cells capable of crossing the BBB such as monocytes, leukocytes, macrophages and CD4+ T-cells. Once these cells are inside the CNS, the virus is released leading to the spread of HIV-1 infection [46].
2) Dysfunction of BBB resulting in direct entry of HIV-1 **(FIG. 4: 1b)**: In vivo studies using HIV-1 gp120 transgenic mice demonstrated that changes in brain endothelium permeability were due to the action of circulating gp120 protein [47]. Gp120 can be extremely toxic to neuronal and brain endothelial cells, chronic exposure to circulating free virus and free viral proteins can lead to BBB disruption, neuronal death and possibly to the development of HAND [48]. Gp120 can increase the expression of matrix metalloproteinases (MMPs) 2 and 9 and decrease key constituents of the BBB, laminin and claudin-5, resulting in disruption of the BBB tight junctions and consequent increase in permeability. It was also shown that gp120 brings about these changes through mechanisms involving ROS generation and oxidant injury, made evident by the increase in MDA levels, a sign of excessive lipid peroxidation, and the reduction of gp120-induced oxidative stress following gene delivery of antioxidant enzymes [49]. Other viral proteins such as Tat, Vpr and Nef also contribute to this breakdown of the BBB [50,51].

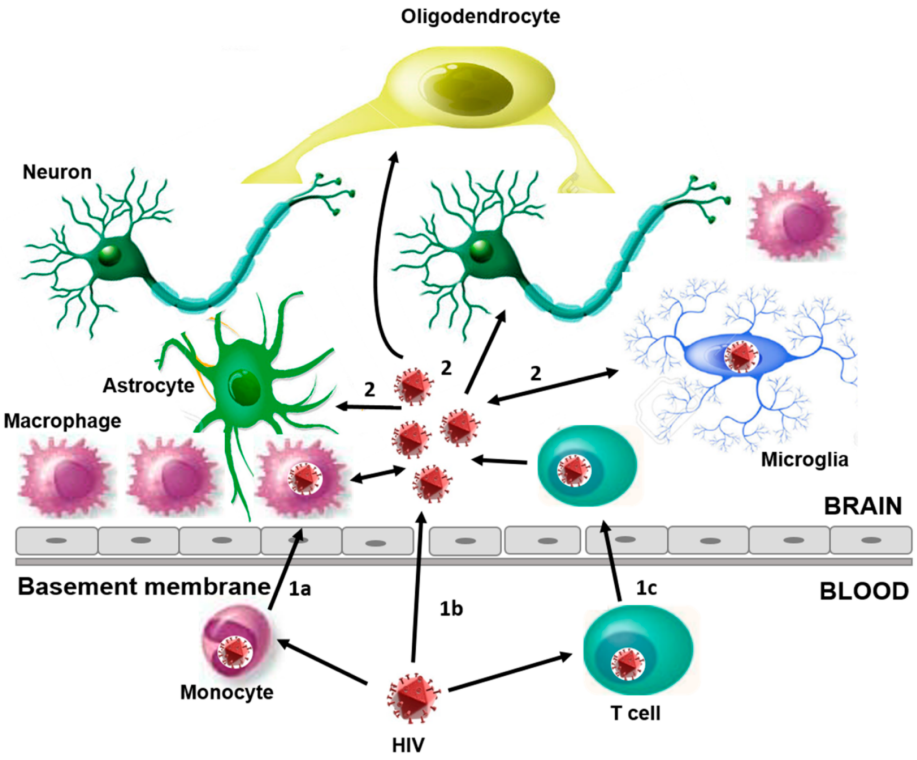

FIGURE 4: PATHWAYS USED BY HIV-1 TO INDUCE CNS TOXICITY. (1a/c) the "trojan horse" mechanism, infected cells cross BBB and once inside liberate virus; **(1b)** direct entry through a dysfunctional and more permeable BBB as a result of virus induced damage to membrane; **(2)** CNS resident cells- microglia, neurons, astrocytes, oligodendrocytes are infected by HIV-1which promotes release of pro-inflammatory cytokines. This causes further destruction of CNS tissue, promoting further neuro-invasion by HIV-1and other microorganisms (increased risk of opportunistic infections).
(Adapted from [45])

The **indirect pathway** involves the multiple ways that HIV-1 can alter the CNS to bring about pathological changes:

1) Infection of resident CNS cells **(FIG. 4: 2):** Each cell type plays an important role in the establishment of viral infection within the CNS (**TABLE 1**). For example, macrophages have the ability to resist the virus' cytopathic effects, the virus can remain inside these cells for an indefinite amount of time. Persistence of the virus leads to alterations in brain structure and function- progressive neuronal loss- cortical and subcortical volume reductions, white matter changes and metabolite abnormalities have all been reported [52].

TABLE 1: ROLE OF CNS CELLS IN HIV-1 INDUCED CNS TOXICITY

Cell Type	Effects in the CNS
Astrocyte	- Increases BBB permeability - Promotes migration of monocytes into the brain - Increases production of neurotoxins
Microglia	- Induces the release of viral proteins (gp120, Tat, Vpr) - Induces neurotoxins production - Activates viral replication
Neuron	- Increases the release of intracellular Ca^{2+} - Increases caspase activation - Increases p53 expression
Oligodendrocyte	- Increases cellular apoptosis - Reduces myelin synthesis - Increases intracellular Ca^{2+} levels
Macrophage	- Induces the release of viral proteins (gp120, Tat, Vpr) - Induces the neurotoxins production - Activates viral replication

(Adapted from [45])

2) Release of viral proteins by infected cells- The viral proteins that trigger neurotoxicity are gp120, Tat and Vpr **(TABLE 2)**. Tat can induce profound proinflammatory effects in the brain through the upregulation of inflammatory cytokines. It can also increase the amount of adhesion molecules facilitating monocyte adhesion and migration into the CNS [53]. In astrocytes, increased expression of monocyte chemoattractant protein-1 (MCP-1) is mediated by NF-κB [54]. The same was also observed in brain endothelial cells [55]. One of the main ways that damage is induced is through the generation of ROS, which leads to the exhaustion of the antioxidant defence system and decreased cell viability. Gp120 and Tat can significantly decrease levels of antioxidants such as GSH, GPx and GR leading to increase in MDA levels showing that cells were oxidatively challenged. A widely accepted indicator of OS is the GSH/GSSG ratio, which is significantly decreased following cell exposure to gp120 and Tat. Brain endothelial cells are highly susceptible to OS as they are rich in polyunsaturated fatty acids (PUFA) meaning that these cells are more susceptible to lipid peroxidation [27,56]. HIV-1 infection leads to high levels of nuclear and mitochondrial genomic DNA damage in cortex autopsy tissue of HAND patients, however, this was

also present in the tissue samples from patients without HAND symptoms. Superoxide anions were detected in the cerebrospinal fluid of AIDS patients [57].

3) Release of cellular factors from infected cells- Infected macrophages, microglia and astrocytes produce toxic molecules including cytokines, ROS and several neurotoxins that impair cellular function, alter neurotransmitter action and may contribute to neuronal loss. There are various cytokines released during HIV-1 infection of the CNS, the ones that are strongly associated with pathogenesis of neurological dysfunction are TNF- α and to a lesser extent IL-6 and IL-10. Oligodendrocytes are particularly sensitive to the effects of TNF- α [54].

TABLE 2: EFFECTS OF VIRAL PROTEINS IN THE CNS

Viral Protein	Effects in CNS
Tat	- Upregulates mRNA levels of inflammatory genes - Upregulates MCP-1 protein expression - Upregulates TNF- α protein expression - Potentiates cytotoxicity of TNF- α - Upregulates expression of adhesion molecules (ICAM-1 and VCAM-1) - Facilitates monocyte infiltration - Increases presence of activated microglial cells - ROS generation
Gp120	- Increases expression of MMP2/9 - Decreases expression of Laminin/ claudin-5 - ROS generation
Vpr	- Stimulates the release of TNF- α, IL-1β, and IL-8 in macrophages - Induces the release of neurotoxins (matrix metalloproteinases) - Promotes cell-cycle and pro-apoptotic proteins

(Adapted from [54])

2.2.4 CARDIOVASCULAR DISEASE AND LUNG PATHOLOGIES

Cardiovascular disease (CVD) and lung pathologies are quite common among individuals infected with HIV-1. Many of these patients are more likely to have risk factors for the development of these diseases, but it also seems that the virus itself is responsible for inducing some of the pathological changes observed in these patients. HIV-1 is able to affect many aspects of the cardiovascular system such as the pericardium, myocardium, coronary arteries, pulmonary vasculature and valves, as well as the systemic vasculature. The involvement of these areas leads to the development of diseases that include pulmonary artery hypertension, HIV-associated cardiomyopathy and pericardial disease [58].

In relation to the lungs, various diseases of the airways (asthma, emphysema, bronchiectasis), lung parenchyma, pulmonary vasculature and certain malignancies, have all been associated with HIV-1 infection [59].

The development of many of these diseases is believed to be promoted by virus-induced OS. This is particularly evident in the genesis of atherosclerosis where HIV-1 alone is capable of accelerating this process. The interaction of HIV-1 with host immune cells (T-cells and macrophages) and with endothelial cells, is responsible for the chronic induction of molecular processes involved in atherogenesis. The major player seems to be the macrophage. HIV-infected macrophages trigger several molecular pathways that induce OS, endoplasmic reticulum stress, inflammasome formation and dysregulation of autophagy **(FIG. 5)** [60]. In the lung, OS and alterations to basal inflammatory status has been linked to Tat overproduction. Tat-transgenic mice display increased expression of OS genes and also raised oxidative indices. Activation of NF-κB is thought to mediate some of the cellular processes leading to OS. Raised levels of MnSOD were also seen, this could be the body's attempt to counteract the OS. These mice also displayed increased cellular infiltration but overall lung morphology was not affected [61].

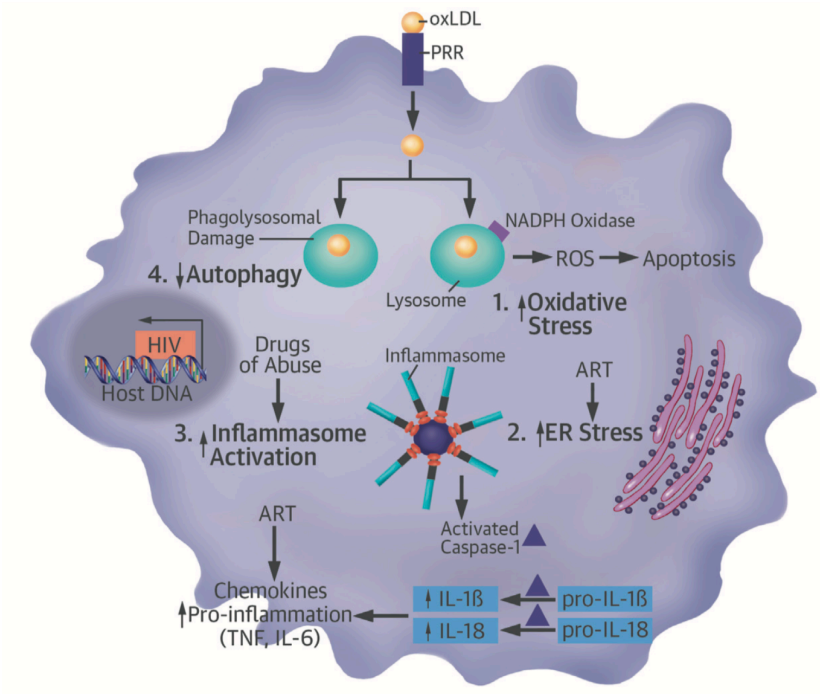

FIGURE 5: MOLECULAR MECHANISMS IN HIV-INFECTED MACROPHAGE CONTRIBUTING TO ATHEROGENESIS. (1) Increased OS; (2) Increases ER stress; (3) Increased inflammasome activation and cytokine production; (4) Decreased autophagy. NADPH= nicotinamide adenine dinucleotide phosphate; oxLDL= oxidised low-density lipoprotein; PRR= pathogen recognition receptor.
(Adapted from [60])

3. OS AND ANTIRETROVIRAL THERAPY (ART)

There are currently 6 groups of antiretrovirals used in the treatment of HIV/AIDS [62]:

- Nucleoside reverse transcriptase inhibitors (NRTIs)
- Nucleotide reverse transcriptase inhibitors (NtRTIs)
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
- Protease inhibitors (PIs)
- Fusion inhibitors (FIs)
- Integrase inhibitors (INIs)/ Co-receptor inhibitors (CRIs)

Highly active antiretroviral therapy (HAART) refers to the combined administration of at least 3 antiretroviral drugs from at least 2 different classes. The first line combination is 2 NRTIs and 1 NNRTI. This helps increase their efficacy and decrease emergence of resistant strains [63]. However, these drugs are associated with many adverse effects [64], and it is thought that many, if not all, of the pathologies induced by antiretroviral drugs are the result of free radical damage to cells [62].

HIV-1 infection increases OS and HAART can further amplify this, not only that but the magnitude of OS depends on the composition of the therapeutic regimen. This was shown by Ngondi et al. who administered different combination therapies to patients and noticed that there was an overall increase in lipid peroxidation among treated patients. However, ART was able to reduce oxidative attack on proteins as shown by the decrease in carbonyl. One of the combinations was linked to a decrease in vitamin C [65]. Total antioxidant status (TAS) was significantly lower in HIV-1 patients undergoing HAART [66], which could suggest that some ART could make patients more susceptible to oxidative damage and that they could potentially benefit from nutritional supplementation.

Mitochondrial damage as a result of HAART has also been linked to the excessive OS observed. ART are unable to discriminate nuclear DNA (their target) from mitochondrial DNA, meaning that this organelle suffers the effects of ART. NRTIs inhibit mitochondrial DNA polymerase gamma (DNA pol-γ), a very important enzymes needed for the replication and repair of mitochondrial DNA. This results in depletion of mitochondrial DNA, altered oxidative phosphorylation and decreased ATP production, all of which contribute to augmented ROS production and consequent cellular damage [63].

4. ANTIOXIDANTS

The chronic use of HAART and HIV-1 itself leads to a state of OS within the body as a result of imbalance between ROS generation and antioxidant activity. Impaired antioxidant defence mechanisms have been reported in HIV-positive patients, mainly depletion of GSH in plasma, lymphocytes, monocytes and lung epithelial lining [25]. This oxidative environment is toxic for cells and can greatly impact their ability to function correctly, leading to weakened immune defences and the development of a multitude of diseases in HIV-infected patients.

Micronutrients such as vitamin A, B-complex, C, and E and selenium and zinc are all required for the adequate functioning of the immune system, cellular differentiation, enzymatic processes, among many others. Micronutrient deficiencies appear to be common among individuals living with HIV/AIDS. Lack of these essential micronutrients may contribute to excessive OS accelerating immune cell death and increased rates of HIV-1 replication [67,68]. Micronutrient deficiencies seem to be more common in less developed countries were adequate HIV-1 therapy and nutrition are not always possible. Akiibinu et al. measured plasma levels of cobalt (Co), copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), selenium (Se), vitamin C, vitamin E, total antioxidant potential (TAP), total plasma peroxide (TPP), oxidative stress index (OSI) and malondialdehyde (MDA) in Nigerian patients. They reported that plasma levels of TAP, Cu, Zn, Fe, Co, Se, vitamin C and vitamin E were significantly lower in symptomatic HIV-positive/AIDS patients when compared with the controls and that the mean values of MDA, TPP and OSI increased significantly in symptomatic HIV-positive/AIDS patients. Fe, Zn, Se, Cu, Mn are essential for the correct functioning of multiple antioxidant enzymes that protect the cells against highly toxic ROS and also to enhance the immunologic activities of phagocytes and lymphocytes. It was concluded that symptomatic HIV-positive/AIDS patients demonstrated elevated oxidative stress and that micronutrient supplementation could be beneficial in these patients to help manage disease and decrease the risk of developing metabolic and immune disturbances [69]. Similar situations have also been observed in children infected with HIV, levels of MDA were significantly higher than in controls, highlighting the importance of oxidative stress in the pathophysiology of this infection [70].

It has also been shown that polymorphisms in certain genes could be the cause of this altered redox state. Recently a study was conducted into the effects of GSTM1 null-allele polymorphism on oxidative stress and disease progression in HIV-1 infected individuals. GSTM1 gene codes for antioxidant enzyme Glutathione S-transferase (GST); those with the GSTM1 genotype had higher CD4 cell count, lower HIV-1 viral load, lower rates of apoptosis and lower OS biomarkers such as 8-oxo-dG when compared to GSTM1 null-allele polymorphism. The GSTM1 null-allele polymorphism is more common among Caucasians and has been linked to reduced mitochondrial activity, decreased detoxifying capacities, increased levels of ROS and increased risk of diseases such as diabetes mellitus, certain cancers and other age-related diseases. This study also implied that there is potential for GSTM1 to be used as a clinical marker to quantify risk for oxidative damage in HIV-infected individuals, possibly serving as a criterion to demonstrate a need for antioxidant supplementation [71].

The use of anti-retroviral medication has greatly extended and enhanced quality of life in individuals infected with HIV, but with it comes the health problems associated with the therapy itself and as part of the normal ageing process. However, in many parts of the world access to these life changing treatments is not always possible due to elevated costs. It is important to find a low-cost intervention that can be used as an adjuvant or as a stand-alone treatment to help attenuate these problems and aid in the slowing of disease progression. Nutritional interventions, such as antioxidants or multivitamin supplementation could potentially be this inexpensive method.

Multiple trials have been conducted analysing the use of multivitamins in HIV-1 patients. Fawzi et al. showed that a multivitamin consisting of vitamin B complex, vitamin C, and vitamin E significantly delayed the progression of disease among HIV-infected women [72]. Another was conducted in Botswana, sub-Saharan Africa, an area that reports one of the highest rates of HIV-1 infection in the world. HIV-1 subtype C is highly prevalent and is associated with more prolonged early viremia and adverse health consequences. A multivitamin composed of thiamine 20 mg, riboflavin 20 mg, niacin 100 mg, vitamin B_6 25 mg, vitamin B_{12} 50 µg, folic acid 800 µg, vitamin C 500 mg and vitamin E 30 mg was given during 24 months to ART-naïve HIV-infected adults, a significant reduction in risk of immune decline and morbidity was observed. It was suggested that micronutrient supplementation may be more effective if initiated at an early stage of HIV-1 disease [73].

However, these promising results are not always evident. In a study by Guwatudde et al. multivitamin supplementation did not have an effect on indicators of disease progression among HIV-1 infected adults initiating HAART. The participants in this study were relatively well-nourished which could explain why an effect was not seen [74]. A systemic review by Visser et al. showed that a supplementation with multiple micronutrients had little or no effect on reducing death in people living with HIV-1 or in disease progression as measured by CD4 cell count and HIV-1 viral load. Then again, intake of vitamin A, D, zinc, or selenium could improve blood levels of each vitamin especially if patient had low levels before supplementation [75].

It seems that the deficiencies observed in HIV-positive patients are probably due to increased utilization of antioxidant micronutrients as a result of increased OS. Even though there are conflicting results, it is extremely important for people living with HIV-1 to have an adequate dietary intake of vitamins and other beneficial compounds. Micronutrient intakes at daily recommended levels need to be assured, either through diet or through the use of supplements. It is crucial that these patients do not suffer from malnutrition, as this could lead to further weakening of the immune system and increase susceptibility to often fatal opportunistic infections [76]. In the next section, we shall explore specific antioxidative compounds and their anti-viral properties.

4.1 N-ACETYLCYSTEINE (NAC)

NAC is a cysteine prodrug and a direct precursor of GSH synthesis. GSH is a very important antioxidant with a major role in regulating T-cell immune function. Consumption of GSH is high during HIV-1 infection and deficiency of this antioxidant has been linked to reduced CD4+ T-cell count and decreased survival in HIV-infected patients [77]. Oral NAC administration in a randomized, 8-week

double-blind, placebo-controlled trial showed that whole blood GSH and T cell GSH levels were safely replenished in HIV-infected individuals [78]. Oral administration of NAC at doses up to 8000 mg/day is not known to cause clinically significant adverse reactions [79]. Data also exists that shows that NAC can prevent many of the consequences bought about by viral proteins. In the presence of NAC, Tat is unable to induce ROS production or DNA damage [26]. Exogenous addition of GSH or of its prodrug, NAC, protected budding yeasts from Vpr-induced cytopathic effects [25]. A study observing whether NAC could counteract Vpr-induced oxidative stress in astroglioma cells obtained positive results [26]. NAC has a direct influence on the NF-κB pathway, by decreasing its activity it can lower levels of apoptosis [27] and alter cytokine release during inflammation [80]. NAC can react rapidly with free radicals, replenish intracellular GSH and reduce oxidant-induced cell damage, suggesting that there may be a clinical role for NAC therapy in protecting against OS and improving immune function in these patients.

4.2 VITAMIN A

Vitamin A is a group of fat-soluble compounds that are stored in the liver and can influence epithelial, reproductive and immune function. Within the immune system, vitamin A is essential, it regulates the production of white blood cells such as lymphocytes, cells that are indispensable in the fight against pathogens. Two forms of vitamin A are available in the human diet: preformed vitamin A (retinol and its esterified form, retinyl ester) and provitamin A carotenoids. Preformed vitamin A is found in dairy products, fish and meat (especially liver). The most important provitamin A carotenoid is beta-carotene, found in high concentrations in colourful fruit and vegetables. Both provitamin A and preformed vitamin A must be metabolized intracellularly to retinal and retinoic acid (active forms of vitamin A) to be able to carry out the vitamin's important biological functions. A plasma retinol concentration lower than 0.70 micromoles/L reflects vitamin A inadequacy. Pregnant women and children have the highest rates of vitamin A deficiency because these populations have increased needs for this micronutrient [81].

Studies have shown that a significant proportion of HIV-1 infected individuals have vitamin A deficiency. Low serum levels or low intakes of vitamin A in HIV-1 infection have been associated with lower CD4+ cell counts, increased maternal-fetal transmission, increased mortality from AIDS or infections, and increased risk of progression to AIDS [82]. Pregnant women with low serum levels of vitamin A have a 3- to 4-fold increased risk of mother-to-child transmission (MTCT). Global data indicates that vitamin A deficiency is most prevalent in areas with higher prevalence of HIV-1 and MTCT, including sub-Saharan Africa and Southeast Asia. It has been speculated that vitamin A deficiency may compromise the epithelial integrity of tissues in the vaginal mucosa and mammary glands meaning that there is a higher probability of infant exposure to HIV-1 during vaginal delivery and in breast milk [83,84]. It has been suggested that HIV-1 pregnant women could benefit from vitamin A supplements. However, several clinical trials have documented the lack of a beneficial effect of vitamin A supplementation on reducing the mother-to-child transmission of HIV. In view of these findings, it has been suggested that serum retinol concentrations may be merely markers of HIV-disease severity, which is an important

predictor of vertical transmission [85]. Children with low levels of vitamin A are more likely to have growth problems, respiratory infection, severe diarrhoea and AIDS-related death. Supplementation with vitamin A was shown to be effective in children born to HIV-infected mothers by significantly lowering the overall morbidity among these infants [83,86]. In adult individuals, low blood levels of vitamin A are associated with accelerated disease progression and increased mortality in HIV-infected adults [82]. A study conducted by Visser et al. showed that vitamin A deficiency was more common in more advanced stages of HIV-1 infection. Retinol levels were low in 39 % of patients with early disease (WHO clinical stages I and II) compared with 48 and 79% of patients with WHO stage III and IV respectively. They also showed a link between low retinol levels and low CD4+ cell counts, normalisation of retinol levels was associated with higher counts, suggesting that retinol may be important for the differentiation of CD4+ cells [87].

It could be argued that patients would benefit from targeted micronutrient supplementation. Then again, studies have shown that there is little association between serum vitamin A levels and vitamin A intake from food and/or supplements [82]. It could be that dosage and duration of treatment were not adequate enough to bring about positive results. Also, since vitamin A is a fat-soluble nutrient, levels can accumulate in the body and dose-related toxicities may occur. All these factors require further investigation to be able to reach a consensus on the benefits of vitamin A.

4.3 VITAMIN E AND C

Vitamin E is a lipid soluble vitamin that is exclusively obtained from the diet. There are 8 isomers of vitamin E, α- and γ-tocopherols that are the most abundant within foods. The richest dietary sources of vitamin E are vegetable oils as they contain all the different homologues in varying proportions. Examples include coconut, maize, palm, olive, peanut soybean wheatgerm and sunflower oil. Within the body only α-tocopherol is found in its active form [88]. Vitamin E is highly lipophilic, as a result of this it is found mostly in cell membranes especially in those where free radical production is greatest, such as mitochondrial membrane and endoplasmic reticulum. Vitamin E is capable of protecting vitamin A and other essential fatty acids from oxidation [89].

Vitamin C is also known as ascorbic acid (AA). This water-soluble vitamin has to be obtained exclusively from the diet, the main contributors of vitamin C are citrus fruits, tomatoes and potatoes. Vitamin C is needed for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters; it is also involved in protein metabolism and plays a vital role in wound healing. It can also regenerate other antioxidants within the body, including α- tocopherol [90,91]. Vitamin C is an important physiological antioxidant that can work alone or alongside other antioxidants such as Vitamin E and the carotenoids. As a reducing agent, vitamin C can neutralize ROS such as hydrogen peroxide, protecting against oxidative cell damage [92]. In a study conducted by Allard et al. daily supplementation of 800 IU vitamin E and 1000 mg vitamin C significantly decreased oxidative stress and decreased viral load [93]. Nonetheless, not enough evidence exists demonstrating just how effective vitamin C and E can be in HIV-infected individuals. Further investigations are required to establish therapeutic values and duration of treatment in order to have significant impact in disease progression.

4.4 POLYPHENOLS

Polyphenols are a versatile group of active phytochemicals abundantly present in the human diet. They are naturally occurring compounds found in most fruits, vegetables and cereals. Examples of fruits that include large quantities of polyphenols, up to 200-300 mg polyphenols per 100 grams fresh weight, include grapes, apple, pears, cherries and many berries. Legumes and chocolate also contribute to the polyphenolic intake. Products produced using these ingredients also retain polyphenols, a glass of wine or a cup of tea contains about 100 mg polyphenols [94]. These compounds are of great interest to scientists owing to their observed biological effects in vitro such as free-radical scavenging, modulation of enzymatic activity, and inhibition of cellular proliferation, as well as their potential utility as anti-allergic, anti-diarrheal, anti-ulcer, and anti-inflammatory agents [95]. Additionally, it has been reported that polyphenols are potent antimicrobials with anti-bacterial, anti-fungal and anti-viral activities. They can potentiate the activity of conventional antimicrobial agents and are often effective against multidrug resistant strains [96]. Polyphenols are the most abundant antioxidant in food and it is well established that when polyphenol-rich foods and beverages are consumed, the plasma antioxidant capacity increases. Due to this strong antioxidant property polyphenols studies suggest that long-term consumption of diets rich in plant polyphenols offer protection against the development of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes and osteoporosis [97].

Polyphenols are generally divided into two groups, flavonoids and non-flavonoids.

Flavonoids are ubiquitously present in all photosynthesizing cells and more than 4000 naturally occurring flavonoids have been identified, the majority being in fruits, vegetables and plant-derived beverages, such as tea and wine. Based on the general properties of polyphenols, it has been suggested that they can be used as therapeutic agents in HIV-1 infection to help protect against the various symptoms that occur as part of disease progression- opportunistic infections, cancers, HIV-mediated neurodegenerative conditions, clinical manifestations as consequence of HIV-mediated oxidative stress and weakened immune system. This can be mediated through their antioxidant capacity, promoting a state of redox balance within cells or through their ability to inhibit various molecular targets involved in HIV-1 replication [98]. The flavonoids can be further divided into the subclasses: flavonols, flavones, flavanols, isoflavones, flavanones, chalcones and anthocyanidins.

Flavonols are found in onions, bananas, apples, tea and red wine. Quercetin and kaempferol are the most abundant members of this group and they have been shown to inhibit syncytium formation, protect against HIV-1 induced cytopathic effects and directly inhibit viral reverse transcriptase (RT) and viral enzyme integrase (IN).

Flavones such as luteolin can inhibit HIV-1 infection in primary human lymphocytes, abolish HIV-1 Tat-driven long terminal repeat (LTR) promoter transactivation by inhibiting Tat binding also prevents NF-κB activation and inhibits host factors involved in transcription [98].

Flavanols are commonly called catechins (C). Catechins are the main constituents of green tea; accounting for up to 30-40%% of the dry weight of green tea leaves are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) [99]. These

green tea catechins possess anti-viral activity by protecting against HIV-1 infection at multiple points during its infective life cycle: they can inhibit sexual transmission of the virus, target key proteins of HIV-1replicative machinery and are capable of modifying host cell factors to impede viral replication [97]. All four catechins inhibit HIV-1 integrase effectively, it seems that they reduce the activity of HIV-1 integrase by disrupting its interaction with virus DNA. Since they have different affinities for different parts of the enzyme, it has been suggested that the combined use of all four will inhibit integrase function more thoroughly [100].

Among the green tea catechins, EGCG is the most abundant and effective compound against HIV-1infection and it seems that the presence of the galloyl moiety is largely responsible for this [99]. EGCG has multiple modes of action [101].

Firstly, it has been shown that EGCG can affect sexual transmission of HIV-1 by directly supressing the infectivity enhancing effect of semen-derived enhancer of viral infection (SEVI) at non-toxic concentrations. SEVI are fibrils that capture HIV-1 virions and direct them to target cells, where they facilitate the fusion of virus and host cells. EGCG can either interfere with de novo SEVI formation or lead to degradation of already formed fibrils without causing any cytotoxicity [102].

Secondly, when present at physiological concentrations EGCG can reduce the attachment of gp120 to CD4+ by a factor of between 10- and 20-fold. Without this interaction, HIV-1 is unable to infect CD4+ cells [103].

Thirdly, EGCG treatment, both in vitro and in vivo, is capable of attenuating neuronal damage induced by viral coat proteins, gp120 or Tat. HIV-1 can be neurotoxic and lead to the development of HIV-associated dementia (HAD). Gp120 and Tat activate JAK/STAT signalling leading to the activation of cell death pathways; this activation is further enhanced in the presence of inflammatory cytokine, IFN-γ [104].

Fourthly, EGCG acts as an allosteric inhibitor of reverse transcriptase (RT) but through a different mechanism than currently approved NNRTIs. It was shown that EGCG exerted synergistic effects when combined with azidothymidine (AZT), allowing for a more effective control over the viral replication [105]. Furthermore, EGCG has the ability to inhibit p24 antigen production in a dose-dependent manner in freshly isolated human CD4+ T-cells and macrophages infected by a wide range of HIV- 1 clinical isolates and laboratory subtypes B, C, D and G [106,107].

Theaflavins are found in brewed black tea and are formed by oxidative dimerization of various green tea polyphenols such as EC, EGC, ECG and EGCG. Yang et al. showed that an economic natural preparation containing 90% of theaflavins (TFmix) inhibited HIV-1 envelope protein-mediated cell-cell fusion, targeted gp41 preventing gp41 six-helix bundle (6-HB) formation and that TFmix could also hinder RT activity but at concentrations 8-fold higher than those needed to inhibit gp41. It was found that TFmix strongly inhibited infection by a broad spectrum of HIV-1 strains, including those resistant to HIV-1 fusion/entry inhibitors. TFmix is stable in acidic conditions and has low cytotoxicity, meaning that theaflavins could be developed as affordable anti-HIV-1 agents and help prevent sexual transmission of HIV-1[108].

The most prominent members of the isoflavones are genistein and daidzein, both abundant in soybeans. Adams et al. demonstrated that primary cortical cell cultures exposed to genistein or daidzein showed an attenuation of Tat induced expression of apoptotic proteins and subsequent cell death. They did this by preventing upregulation of caspase activity and by blocking the induction of pro-apoptotic proteins Bax and Bcl-2 [109]. Genistein also appears to have an inherent ability to inhibit tyrosine kinase and to prevent HIV-1 infection of primary macrophages [99].

The most common **non-flavonoids** are phenolic acids, these include caffeic acid found in fruits, vegetables and coffee and ferulic acid mostly found in cereals [95]. Resveratrol (RSV) belongs to the stilbenes subgroup. Fresh grape skin contains about 50-100 g of RSV per gram wet weight thereby contributing relatively high concentration of resveratrol to the products prepared from grapes such as red wine and grape juice [97]. RSV can attenuate superoxide generation in the mitochondria and reduce mitochondrial dysfunction. It can also scavenge for $O_2^{\bullet-}$ and OH· in vitro and prevent glutathione depletion. However, these properties are dose dependent as it seems that elevated levels of RSV can actually promote an increase in ROS production [110]. RSV can also prevent some of the side effects associated with ART. Protease inhibitors (PIs) have been linked to cardio-metabolic complications such as type-2 diabetes and coronary heart disease in HIV-infected patients. Symington et al. showed that PI treatment significantly lowered body weight and cardiac respiratory function and that co-treatment with RSV for 4 months attenuated the PI-mediated decrease in body weight and enhanced cardiac mitochondrial respiratory capacity in PI-treated rats [111]. RSV can potentiate the antiretroviral activity of NRTIs in drug-resistant HIV-strains. This was shown in HIV-1 strains carrying the M184V mutation in the HIV-1 reverse transcriptase (RT) gene. Strains of HIV-1 with this mutation are sometimes resistant to emtricitabine (FTC) as well as other NRTIs. Administration of RSV to these FTC- resistant cell lines show that it is capable of inhibiting FTC-resistant and multi-drug resistant viruses [112].

5. CONCLUSION AND FUTURE PERSPECTIVES

Excessive OS has been shown to have a pathological role in HIV-1 infection, contributing to disease progression to AIDS and the development of age- related diseases in younger patients. HIV-1 induces OS and has the capacity to disrupt the antioxidant defence system and initiate various oxidative reactions that result in excessive ROS production. This imbalance in cellular redox state leads to the activation of various pathways that induce cell death.

Viral proteins Tat, Vpr and gp120 bring about this state mostly through their activation of NF-κB. Intracellular Tat in HIV-1 infected CD4+ T-cells is beneficial for the virus as it promotes viral replication, delays Fas-mediated apoptosis and increases infectivity of virus. In mutant strains that lack Tat these processes are greatly impaired. When Tat is released and taken up by adjacent non-infected cells it is toxic, capable of inducing mitochondrial dysfunction, dysregulation of gene expression, ROS production and activation of programmed cell death. All this contributing to the rapid spread of infection, depletion of CD4+ T-cells and progressive deterioration of the immune system. Tat also promotes genetic instability and malignant transformation in B-cells resulting in an increased risk of lymphoma development. Therefore, developing Tat inhibitors could be beneficial to help prevent disease progression and also potentially reduce the occurrence of HIV-1-related lymphomas. Vpr is capable of causing structural and functional damage especially to the mitochondria. Impaired mitochondria are the main contributor to excessive ROS production. It can also increase viral replication in HIV infected CD4+ T-cells and increase infectivity of macrophages. Gp120 is an important protein that mediates many interactions between the virus and its surrounding. This glycoprotein is responsible for the initial infection of CD4+ T-cells by HIV-1, it has been implicated in HIV-1- induced BBB damage and resulting neurotoxicity. It also promotes apoptosis through the increased expression of pro-apoptotic molecules such as Fas and FasL. The transcription factor, NF-κB, controls a multitude of cellular pathways, mainly those that promote a pro-oxidative state. In latent macrophage cells, NF-κB have to be kept low to prevent cell death from occurring and allow HIV-1 to remain inside. It has also been shown that different HIV-1 subtypes display different numbers of NF-κB binding sites, suggesting that some sub-types are more susceptible to the actions of NF-κB than others.

Through the interplay of the various viral proteins mentioned, the main consequence is the induction of cell death of CD4+ T-cells which for the virus is highly advantageous. CD4+ T-cells are proliferating but at a lesser rate compared to the virus, this means that the virus is able to infect and cause the death of these cells faster than they can regenerate. By overwhelming the immune system's regenerative capacity, viral replication can go unchecked and infection levels can rise. Depletion of CD4+ T-cells greatly weakens the immune system and with time AIDS develops. HIV-1 has many ways in which it can gain entry into the CNS to induce toxicity. Once inside it causes toxicity mainly through the action of gp120. Tat, Vpr and Nef also contribute to this process. Lastly, HIV-1-induced OS promotes damage to the CVS and to the lungs.

HAART is essential to actively control viral replication and allow patients to live longer and with better quality of life. It seems that these medications also contribute to OS in their own way and therefore

potentiate some of the damaging effects induced by HIV-1. Not all drugs contribute to this process in the same way, making it extremely important that the drugs chosen are those that are capable of controlling the infection and at the same time decrease likelihood of promoting damaging effects.

The decrease in antioxidants that accompanies HIV-1 infection suggests that nutritional supplementation and the promotion of a good nutrition in general could help with the management of HIV/AIDS. The addition of antioxidants to the therapeutic regimen of these patients could potentially prevent the additional damage caused by the free radicals generated by the infection itself and by HAART. There are many positive results surrounding the use of antioxidants in HIV-1 infected patients. NAC was highly effective at increasing serum antioxidant levels and was also shown that it could inhibit Tat, Vpr and NF-κB. Vitamin A could prove beneficial especially in children and pregnant women. Vitamin E and C can also neutralise ROS and protect from their damaging effects. Polyphenols consists of multiple compounds that could be potentially useful not only in protecting against acute HIV-1 infection but also for the management of HIV/AIDS as a chronic condition. Within this group the tea catechins which are well-known for their health effects and safety could provide a safe and well-tolerated drug. These compounds are also relatively inexpensive meaning that catechins that contain a galloyl moiety could be used especially among low-income populations. These affordable strategies would be able to improve the morbidity and mortality of HIV-infected adults and children across the world. Many of the studies reviewed presented very optimistic results in relation to the use of antioxidants to aid in the fight against HIV-1. However, conflicting evidence does exist with some studies showing that no effects were observed. This suggest that further investigation is essential in order to verify the findings of these studies and to determine the proper antioxidant dosage, duration of treatment, method of administration and at what stage of HIV-1 infection would antioxidant supplementation bring about greater benefits.

To summarise, HIV-1 contributes to a state of excessive OS within the body. This is mediated by multiple viral proteins; Tat, gp120 and Vpr display a greater amount of evidence of their involvement in this process. The damage caused by the excessive OS is linked to CD4+ T-cell depletion and the development of certain pathologies associated with HIV-1 infection of the CNS, CVS and respiratory system. The use of HAART could potentiate these detrimental effects. PLWH could potentially benefit from greater intake of antioxidant rich foods or even antioxidant supplementation. Not everyone has access to treatment right away meaning that patients are exposed for greater periods of time to uncontrolled viral replication and to the negative effects that result from this. Antioxidants can provide a low-cost and long-term strategy to reduce oxidative stress, prevent micronutrient deficiency, and slow down AIDS progression.

REFERENCES

[1] UNAIDS. *Global HIV & AIDS statistics — 2018 fact sheet.* Available from: http://www.unaids.org/en/resources/fact-sheet [Accessed 17 January 2019].

[2] Wing EJ. HIV and aging. *Int J Infect Dis.* 2016 Dec;53:61-68.

[3] UNAIDS. *THE GAP REPORT 2014- People aged 50 and over.* Available from: http://www.unaids.org/sites/default/files/media_asset/12_Peopleaged50yearsandolder.pdf [Accessed 17 January 2019].

[4] McGettrick P, Barco EA, Mallon PWG. Ageing with HIV. *Healthcare (Basel).* 2018 ; 6(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5872224/ [Accessed 14 January 2019].

[5] Baruchel S, Wainberg MA. The role of oxidative stress in disease progression in individuals infected by the human immunodeficiency virus. *J Leukoc Biol.* 1992;52(1):111–4.

[6] Masiá M, Padilla S, Fernández M, Barber X, Moreno S, Iribarren JA, et al. Contribution of Oxidative Stress to Non-AIDS Events in HIV-Infected Patients. *J Acquir Immune Defic Syndr*. 2017;75(2):e36–44

[7] Couret J, Chang TL. Reactive oxygen species in HIV infection. *EC Microbiol*. 2016;3(6):597-604.

[8] Herzenberg LA, De Rosa SC, Dubs JG, Roederer M, Anderson MT, Ela SW, et al. Glutathione deficiency is associated with impaired survival in HIV disease. *Proc Natl Acad Sci U S A.* 1997;94(5):1967-72.

[9] Agarwal A. Oxidants and Antioxidants in the Pathogenesis of HIV/AIDS. *Open Reprod Sci J.* 2011;3(1):154–61.

[10] Gutowski M, Kowalczyk S. A study of free radical chemistry: Their role and pathophysiological significance. *Acta Biochim Pol.* 2013;60:1–16.

[11] Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* 2009;417(1):1-13.

[12] Bigarella CL, Liang R, Ghaffari S. Stem cells and the impact of ROS signaling. *Development.* 2014;141(22):4206-18.

[13] Bedard K, Krause K-H. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. *Physiol Rev.* 2007;87(1):245-313.

[14] Bhattacharyya S, Sinha K CSP. Cytochrome p450s: mechanisms and biological implications in drug metabolism and its interaction with oxidative stress. *Curr Drug Metab*. 2014;15(7):719–42.

[15] Ivanov A V., Valuev-Elliston VT, Ivanova ON, Kochetkov SN, Starodubova ES, Bartosch B, et al. Oxidative Stress during HIV Infection: Mechanisms and Consequences. *Oxid Med Cell Longev.* 2016;2016:8910396.

[16] Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol.* 2014;24(10):R453-62.

[17] Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. *Biochim Biophys Acta.* 2013;1830:3289–303.

[18] Niture SK, Khatri R, Jaiswal AK. Regulation of Nrf2 - An update. *Free Radic Biol Med.* 2014;66:36-44

[19] Poljsak B, Šuput D, Milisav I. Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. *Oxid Med Cell Longev.* 2013;2013:956792.

[20] Meredith LW, Sivakumaran H, Major L, Suhrbier A, Harrich D. Potent inhibition of HIV-1 replication by a tat mutant. *PLoS One*. 2009; 10;4(11):e7769.

[21] Lin M-H, Apolloni A, Cutillas V, Sivakumaran H, Martin S, Li D, et al. A Mutant Tat Protein Inhibits HIV-1 Reverse Transcription by Targeting the Reverse Transcription Complex. *J Virol.* 2015;89(9):4827-36.

[22] Jin H, Li D, Sivakumaran H, Lor M, Rustanti L, Cloonan N, et al. Shutdown of HIV-1 transcription in T cells by Nullbasic, a mutant tat protein. *mBio*. 2016;7(4): pii: e00518-16.

[23] Rodríguez-Mora S, Mateos E, Moran M, Martín MÁ, López JA, Calvo E, et al. Intracellular expression of Tat alters mitochondrial functions in T cells: A potential mechanism to understand mitochondrial damage during HIV-1 replication. *Retrovirology*. 2015;12:1–24. doi:10.1186/s12977-015-0203-3.

[24] El-Amine R, Germini D, Zakharova V V., Tsfasman T, Sheval E V., Louzada RAN, et al. HIV-1 Tat protein induces DNA damage in human peripheral blood B-lymphocytes via mitochondrial ROS production. *Redox Biol.* 2018;15:97-108.

[25] Monroy N, Herrero L, Carrasco L, González ME. Influence of glutathione availability on cell damage induced by human immunodeficiency virus type 1 viral protein R. *Virus Res.* 2016;213:116-23.

[26] Ferrucci A, Nonnemacher MR, Cohen ÉA, Wigdahl B. Extracellular human immunodeficiency virus type 1 viral protein R causes reductions in astrocytic ATP and glutathione levels compromising the antioxidant reservoir. *Virus Res.* 2012;167(2):358-69.

[27] Price TO, Ercal N, Nakaoke R, Banks WA. HIV-1 viral proteins gp120 and Tat induce oxidative stress in brain endothelial cells. *Brain Res.* 2005;1045(1–2):57–63.

[28] Samikkannu T, Ranjith D, Rao KVK, Atluri VSR, Pimentel E, El-Hage N, et al. HIV-1 gp120 and morphine induced oxidative stress: role in cell cycle regulation. *Front Microbiol.* 2015; 6:614. doi: 10.3389/fmicb.2015.00614

[29] Himanshu Vashistha, Mohammed Husain DK& PCS. Tubular Cell HIV-1 gp120 Expression Induces Caspase 8 Activation and Apoptosis. *Ren Fail.* 2009;31(4):303–12.

[30] Shah A, Kumar A. HIV-1 gp120-mediated increases in IL-8 production in astrocytes are mediated through the NF-κB pathway and can be silenced by gp120-specific siRNA. *J Neuroinflammation.* 2010;7:1–6.

[31] Wang T, Zhang X, Li JJ. The role of NF-κB in the regulation of cell stress responses. *Int Immunopharmacol.* 2002;2(11):1509–20.

[32] Jiang G, Dandekar S. Targeting NF-κB Signaling with Protein Kinase C Agonists As an Emerging Strategy for Combating HIV Latency. *AIDS Res Hum Retroviruses.* 2015;31(1):4-12.

[33] Jiang G, Mendes EA, Kaiser P, Sankaran-Walters S, Tang Y, Weber MG, et al. Reactivation of

HIV latency by a newly modified Ingenol derivative via protein kinase Cδ-NF-κB signaling. *AIDS.* 2014;28(11):1555-66.

[34] Petit F, Arnoult D, Viollet L, Estaquier J. Intrinsic and extrinsic pathways signaling during HIV-1 mediated cell death. *Biochimie*. 2003;85(8):795–811.

[35] Doitsh G, Greene WC. Dissecting How CD4 T Cells Are Lost during HIV Infection. *Cell Host Microbe.* 2016;19(3):280-91.

[36] Banki K, Hutter E, Gonchoroff NJ, Perl A. Molecular Ordering in HIV-induced Apoptosis. *J Biol Chem.* 1998;273(19):11944–53.

[37] Heigele A, Joas S, Regensburger K, Kirchhoff F. Increased susceptibility of CD4+ T cells from elderly individuals to HIV-1 infection and apoptosis is associated with reduced CD4 and enhanced CXCR4 and FAS surface expression levels. *Retrovirology*. 2015;12(1):1–14.

[38] Carter CA, Ehrlich LS. Cell Biology of HIV-1 Infection of Macrophages. *Annu Rev Microbiol.* 2008;62:425-43.

[39] Osman A, Bhuyan F, Hashimoto M, Nasser H, Maekawa T, Suzu S. M-CSF Inhibits Anti-HIV-1 Activity of IL-32, but They Enhance M2-like Phenotypes of Macrophages. *J Immunol.* 2014;192(11):5083-9.

[40] Herbein G, Gras G, Khan KA, Abbas W. Macrophage signaling in HIV-1 infection. *Retrovirology.* 2010;7:1–13.

[41] Meltzer B, Dabbagh D, Guo J, Kashanchi F, Tyagi M, Wu Y. Tat controls transcriptional persistence of unintegrated HIV genome in primary human macrophages. *Virology.* 2018;518:241-52.

[42] Zhang X, Zhou T, Frabutt DA, Zheng YH. HIV-1 Vpr increases Env expression by preventing Env from endoplasmic reticulum-associated protein degradation (ERAD). *Virology.* 2016;496:194-202.

[43] Abbas W, Tariq M, Iqbal M, Kumar A, Herbein G. Eradication of HIV-1 from the macrophage reservoir: An uncertain goal? *Viruses*. 2015;7(4):1578–98.

[44] Deanna Saylor, Alex M. Dickens, Ned Sacktor, Norman Haughey, Barbara Slusher, Mikhail Pletnikov, Joseph L. Mankowski, Amanda Brown, David J. Volsky and JCM. HIV-associated neurocognitive disorder — pathogenesis and prospects for treatment. *Nat Rev Neurol.* 2016;12(4):234–48.

[45] Scutari R, Alteri C, Perno CF, Svicher V, Aquaro S. The role of HIV infection in neurologic injury. *Brain Sci.* 2017;7(4). pii: E38.

[46] Albright A V., Soldan SS, González-Scarano F. Pathogenesis of human immunodeficiency virus-induced neurological disease. *J Neurovirol.* 2003;9(2):222–7.

[47] Cioni C, Annunziata P. Circulating gp120 alters the blood-brain barrier permeability in HIV-1 gp120 transgenic mice. *Neurosci Lett.* 2002;330(3):299–301.

[48] Kanmogne GD, Kennedy RC, Grammas P. HIV-1 gp120 proteins and gp160 peptides are toxic to brain endothelial cells and neurons: Possible pathway for HIV entry into the brain and HIV-associated dementia. J *Neuropathol Exp Neurol.* 2002;61(11):992–1000.

[49] Louboutin JP, Agrawal L, Reyes BAS, Van Bockstaele EJ, Strayer DS. HIV-1 gp120-induced injury to the blood-brain barrier: Role of metalloproteinases 2 and 9 and relationship to oxidative stress. *J Neuropathol Exp Neurol.* 2010;69(8):801–16.

[50] Atluri VSR, Hidalgo M, Samikkannu T, Kurapati KRV, Jayant RD, Sagar V, et al. Effect of human immunodeficiency virus on blood-brain barrier integrity and function: an update. *Front Cell Neurosci.* 2015;9:1–10.

[51] Leibrand CR, Paris JJ, Ghandour MS, Knapp PE, Kim WK, Hauser KF, et al. HIV-1 Tat disrupts blood-brain barrier integrity and increases phagocytic perivascular macrophages and microglia in the dorsal striatum of transgenic mice. *Neurosci Lett.* 2017;640:136–43.

[52] Cohen RA, Seider TR, Navia B. HIV effects on age-associated neurocognitive dysfunction: Premature cognitive aging or neurodegenerative disease? *Alzheimers Res Ther.* 2015;7(1):1–10

[53] Pu H, Tian J, Flora G, Lee YW, Nath A, Hennig B, et al. HIV-1 tat protein upregulates inflammatory mediators and induces monocyte invasion into the brain. *Mol Cell Neurosci.* 2003;24(1):224–37.

[54] Minagar A, Shapshak P, Fujimura R, Ownby R, Heyes M, Eisdorfer C. The role of macrophage/microglia and astrocytes in the pathogenesis of three neurologic disorders: HIV-associated dementia, Alzheimer disease, and multiple sclerosis. *J Neurol Sci.* 2002;202(1–2):13–23.

[55] Toborek M, Lee YW, Pu H, Malecki A, Flora G, Garrido R, et al. HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. *J Neurochem.* 2003;84(1):169–79.

[56] Saing T, Lagman M, Castrillon J, Gutierrez E, Guilford FT, Venketaraman V. Analysis of glutathione levels in the brain tissue samples from HIV-1-positive individuals and subject with Alzheimer's disease and its implication in the pathophysiology of the disease process. *BBA Clin.* 2016;6:38–44.

[57] Zhang Y, Wang M, Li H, Zhang H, Shi Y, Wei F, et al. Accumulation of nuclear and mitochondrial DNA damage in the frontal cortex cells of patients with HIV-associated neurocognitive disorders. *Brain Res.* 2012;1458:1–11.

[58] Manga P, Mccutcheon K, Tsabedze N, Vachiat A, Zachariah D. HIV and Nonischemic Heart Disease. *J Am Coll Cardiol.* 2017;69(1):83-91

[59] Staitieh B, Guidot DM. Noninfectious pulmonary complications of human immunodeficiency virus infection. *Am J Med Sci.* 2014;348(6):502–11.

[60] Kearns A, Gordon J, Burdo TH, Qin X. HIV-1–Associated Atherosclerosis: Unraveling the Missing Link. *J Am Coll Cardiol.* 2017;69(25):3084–98.

[61] Cota-Gomez A, Flores AC, Ling XF, Varella-Garcia M, Flores SC. HIV-1 Tat increases oxidant burden in the lungs of transgenic mice. *Free Radic Biol Med.* 2011;51(9):1697–707.

[62] Tabe F, Yanou N, Armel K, Ntso A-S. Oxidative Role of HIV:AIDS: Antiretroviral Drugs and Medicinal Plants with Anti-HIV Activity. *J Dis Med Plants.* 2015;1:68–75. doi: 10.11648/j.jdmp.20150105.11

[63] Smith RL, de Boer R, Brul S, Budovskaya Y, van Spek H. Premature and accelerated aging: HIV or HAART? Front Genet. 2013;3:328.

[64] Nolan D, Reiss P, Mallal S. Adverse effects of antiretroviral therapy for HIV infection: a review of selected topics. *Expert Opin Drug Saf*. 2005;4(2):201-18.

[65] Ngondi JL, Oben J, Forkah DM, Etame LH, Mbanya D. The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon. *AIDS Res Ther*. 2006;3(1):1–7.

[66] Kolgiri V, Patil V, Nagar V. Correlation of total antioxidant status (TAS) with damage in HIV/AIDS patients. *Int J Pharm Pharm Sci*. 2016;8(6):240–4. Available from: https://www.researchgate.net/profile/Vaishali_Kolgiri/publication/304886773_Correlation_of_total_antioxidant_status_TAS_with_DNA_damage_in_HIVAIDS_patients/links/58216c9508ae12715afe407c/Correlation-of-total-antioxidant-status-TAS-with-DNA-damage-in-HIV-AIDS-patients.pdf [acessed 14 Jnauary 2019].

[67] Gedle D. Effects of HIV/AIDS on Micronutrients. *SM J Food Nutri Disord.*. 2015;1(1):1002.

[68] Allard JP, Aghdassi E, Chau J, Salit I, Walmsley S. Oxidative stress and plasma antioxidant micronutrients in with HIV infection. *Am J Clin Nutr*. 1998;67(1):143–7.

[69] Akiibinu MO, Adeshiyan AA, Olalekan AO. Micronutrients and markers of oxidative stress in symptomatic HIV-positive/AIDS Nigerians: A call for adjuvant micronutrient therapy. *IIOAB J.* 2012;3(2):7–11.

[70] Dias BF, Srinivas A. Oxidative stress in HIV positive children. *Int J Res Med Sci.* 2017;5(4):1578-81.

[71] Parsons M, Campa A, Lai S, Li Y, Martinez JD, Greer P, et al. Effect of GSTM1-Polymorphism on Disease Progression and Oxidative Stress in HIV Infection: Modulation by HIV/HCV Co-Infection and Alcohol Consumption. *J AIDS Clin Res*. 2013;4(9). pii: 10002337.

[72] Fawzi WW, Msamanga GI, Spiegelman D, Wei R, Kapiga S, Villamor E, et al. A Randomized Trial of Multivitamin Supplements and HIV Disease Progression and Mortality. *N Engl J Med.* 2004;351(1):23-32.

[73] Baum MK, Campa A, Lai S, Sales Martinez S, Tsalaile L, Burns P, et al. Effect of micronutrient supplementation on disease progression in asymptomatic, antiretroviral-naive, HIV-infected adults in Botswana: A randomized clinical trial. *JAMA.* 2013;310(20):2154–63.

[74] Guwatudde D, Wang M, Ezeamama AE, Bagenda D, Kyeyune R, Wamani H, et al. The effect of standard dose multivitamin supplementation on disease progression in HIV-infected adults initiating HAART: A randomized double blind placebo-controlled trial in Uganda. *BMC Infect Dis.* 2015;15(1):1–10.

[75] Visser ME, Durao S, Sinclair D, Irlam JH SN. Micronutrient supplementation in adults with HIV infection (Review). *Cochrane Database Syst Rev*. 2017; 18;5:CD003650. doi:10.1002/14651858.CD003650.pub4

[76] Hong H, Budhathoki C, Farley JE. Effectiveness of macronutrient supplementation on nutritional status and HIV/AIDS progression: A systematic review and meta-analysis. *Clin Nutr ESPEN.*

2018;27:66–74

[77] Elbini Dhouib I, Jallouli M, Annabi A, Gharbi N, Elfazaa S, Lasram MM. A minireview on N-acetylcysteine: An old drug with new approaches. *Life Sci.* 2016;151:359–63.

[78] De Rosa SC et al. N-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest.* 2000;30:915–29.

[79] Atkuri KR, Mantovani JJ, Herzenberg LA, Herzenberg LA. N-Acetylcysteine-a safe antidote for cysteine/glutathione deficiency. *Curr Opin Pharmacol.* 2007;7(4):355–9.

[80] Oka SI, Kamata H, Kamata K, Yagisawa H, Hirata H. N-Acetylcysteine suppresses TNF-induced NF-κB activation through inhibition of IκB kinases. *FEBS Lett.* 2000;472(2–3):196–202

[81] National Institutes of Health- Office of Dietary Supplements . *Vitamin A- Fact Sheet for Health Professionals.* Available from: https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/ [Accessed 14 January 2019]

[82] Tang AM, Graham NMH, Semba RD, Saah AJ. Association between serum vitamin A and E levels and HIV-1 disease progression. *AIDS.* 1997;11(5):613–20.

[83] Humphrey JH, Iliff PJ, Marinda ET, Mutasa K, Moulton LH, Chidawanyika H, et al. Effects of a Single Large Dose of Vitamin A, Given during the Postpartum Period to HIV-Positive Women and Their Infants, on Child HIV Infection, HIV-Free Survival, and Mortality. *J Infect Dis.* 2006;193(6):860–71.

[84] McHenry M, Apondi E, Vreeman R. Vitamin A supplementation for the reduction of the risk of mother to child transmission of HIV. *Expert Rev Anti Infect Ther*. 2015;13(7):821–4.

[85] Wiysonge C, Ndze V, Kongnyuy E, Shey M. Vitamin A supplements for reducing mother-to-child HIV transmission. *Cochrane Database Syst Rev*. 2017;2017(9):CD003648.

[86] Coutsoudis A, Bobat RA, Coovadia HM, Kuhn L, Tsai WY, Stein ZA. The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. *Am J Public Health.* 1995;85(8):1076–81.

[87] Visser ME, Maartens G, Kossew G, Hussey GD. Plasma vitamin A and zinc levels in HIV-infected adults in Cape Town, South Africa. *Br J Nutr*. 2003;89(4):475–82.

[88] National Institutes of Health- Office of Dietary Supplements . *Vitamin E- Fact Sheet for Health Professionals.* Available from: https://ods.od.nih.gov/factsheets/VitaminE-HealthProfessional/ [Accessed 14 January 2019].

[89] Saliha Rizvi, Syed T. Raza, Faizal Ahmed, Absar Ahmad, Shania Abbas FM. The Role of Vitamin E in Human Health and Some Diseases. *Sultan Qaboos Univ Med J.* 2014;14(2):e157-65.

[90] Iqbal K, Khan A, Muzaffar Ali Khan Khattak M. Biological Significance of Ascorbic Acid (Vitamin C) in Human Health-A Review. *Pakistan Journal of Nutrition.* 2004;3:5-13.

[91] National Institutes of Health- Office of Dietary Supplements. *Vitamin C- Fact Sheet for Health Professionals.* Available from: https://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/ [Accessed 14 January 2019].

[92] Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: *Current state. Nutr J* . 2016;15(1):71. doi: 10.1186/s12937-016-0186-5.

[93] Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE, et al. Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. *AIDS.* 1998;12:1653–9. doi:10.1097/00002030-199813000-00013.

[94] Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary Polyphenols and the Prevention of Diseases. *Crit Rev Food Sci Nutr.* 2005;45(4):287-306.

[95] Hollman PCH, Katan MB. Dietary flavonoids: Intake, health effects and bioavailability. Food Chem Toxicol. 1999;37(9–10):937–42.

[96] Daglia M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol.* 2012;23(2):174–81.

[97] Pandey K, Rizvi S. Current Understanding of Dietary Polyphenols and their Role in Health and Disease. Curr Nutr Food Sci. 2009;5(4):249–63.

[98] Andrae-Marobela K, Ghislain FW, Okatch H, Majinda RRT. Polyphenols: A Diverse Class of Multi-Target Anti-HIV-1 Agents. *Curr Drug Metab.* 2013;14(4):392-413.

[99] Date AA, Destache CJ. Natural polyphenols: Potential in the prevention of sexually transmitted viral infections. *Drug Discov Today.* 2016;21(2):333–41.

[100] Jiang F, Chen W, Yi K, Wu Z, Si Y, Han W, et al. The evaluation of catechins that contain a galloyl moiety as potential HIV-1 integrase inhibitors. *Clin Immunol*. 2010;137(3):347–56.

[101] Kaihatsu K, Yamabe M, Ebara Y. Antiviral Mechanism of Action of Epigallocatechin-3-O-gallate and Its Fatty Acid Esters. *Molecules*. 2018;23(10):2475.

[102] Hartjen P, Frerk S, Hauber I, Matzat V, Thomssen A, Holstermann B, et al. Assessment of the range of the HIV-1 infectivity enhancing effect of individual human semen specimen and the range of inhibition by EGCG. *AIDS Res Ther.* 2012;9(1):2. doi: 10.1186/1742-6405-9-2

[103] Williamson MP, McCormick TG, Nance CL, Shearer WT. Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: Potential for HIV-1 therapy. *J Allergy Clin Immunol.* 2006;118(6):1369–74.

[104] Giunta B, Obregon D, Hou H, Zeng J, Sun N, Nikolic V, et al. EGCG mitigates neurotoxicity mediated by HIV-1 proteins gp120 and Tat in the presence of IFN-γ: Role of JAK/STAT1 signaling and implications for HIV-associated dementia. *Brain Res.* 2006;1123(1):216–25.

[105] Li S, Hattori T, Kodama EN. Epigallocatechin gallate inhibits the HIV reverse transcription step. *Antivir Chem Chemother.* 2011;21(6):239-43.

[106] Nance CL, Siwak EB, Shearer WT. Preclinical development of the green tea catechin, epigallocatechin gallate, as an HIV-1 therapy. *J Allergy Clin Immunol.* 2009;123(2):459–65.

[107] Sodagari HR, Bahramsoltani R, Farzaei MH, Abdolghaffari AH, Rezaei N, Taylor-Robinson AW. Tea Polyphenols as Natural Products for Potential Future Management of HIV Infection - an overview. *J Nat Remedies*. 2016; 18;16(2):60-72.

[108] Yang J, Li L, Tan S, Jin H, Qiu J, Mao Q, et al. A natural theaflavins preparation inhibits HIV-1 infection by targeting the entry step: Potential applications for preventing HIV-1 infection.

Fitoterapia. 2012;83(2):348–55.

[109] Adams SM, Aksenova M V., Aksenov MY, Mactutus CF, Booze RM. Soy isoflavones genistein and daidzein exert anti-apoptotic actions via a selective ER-mediated mechanism in neurons following HIV-1 Tat1-86exposure. *PLoS One.* 2012;7(5):38–40.

[110] Abba Y, Hassim H, Hamzah H, Noordin MM. Antiviral Activity of Resveratrol against Human and Animal Viruses. *Adv Virol.* 2015;2015:184241. doi: 10.1155/2015/184241

[111] Symington B, Mapanga RF, Norton GR, Essop MF. Resveratrol co-treatment attenuates the effects of HIV protease inhibitors on rat body weight and enhances cardiac mitochondrial respiration. *PLoS One.* 2017 Jan 20;12(1):e0170344

[112] Heredia A, Davis C, Amin MN, Le NM, Wainberg MA, Oliveira M, et al. Targeting host nucleotide biosynthesis with resveratrol inhibits emtricitabine-resistant HIV-1. *AIDS.* 2014;28(3):317–23.