

**ANTIDYSLIPIDEMIC PROPERTIES OF *OCIMUM GRATISSIMUM*  
(ANCHABI) ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY  
ADMINISTERED ALBINO WISTAR RATS**

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**ABSTRACT**

**Background:** Highly active antiretroviral therapy is considered toxic and has other life- threatening side effects including dyslipidemia. The *Ocimum gratissimum* and related species are used in folk medicine for various human diseases. In this study defense property of *Ocimum gratissimum* (Anchabi) over highly active antiretroviral therapy drugs induced dyslipidemia has been evaluated on albino wistar rats.

**Methods:** Thirty six rats of same age and 150-200 g weight were

selected and divided into six groups containing each six. The animals were treated with highly active antiretroviral therapy drugs and different concentration of hydroethanolic leaves extract of *Ocimum gratissimum* (100, 200, 300 and 400 mg/kg) for thirty days. After thirty days treatment, the rats were fasted overnight (12 to 14 hours). The animals were anaesthetized and blood sample was collected by cardiac puncture for biochemical study.

**Results:** Elevated levels of total cholesterol, triacylglycerol, LDL-cholesterol and decreased levels of HDL cholesterol were detected in highly active antiretroviral therapy administered groups. The oral administration of varying doses of hydroethanolic leaves extract of *Ocimum gratissimum* for the period of thirty days reversed these altered results to normal levels in a dose dependant manner. **Conclusion:** These results indicated that *Ocimum gratissimum* efficiently protected against dyslipidemia induced by highly active antiretroviral therapy drugs in rats, possibly through the antioxidative and antidyslipidemic efficacy of *Ocimum gratissimum*.

**KEYWORDS:** *Ocimum gratissimum*, dyslipidemia and highly active antiretroviral therapy.

## INTRODUCTION

Treatment of HIV infection and AIDS is complicated by the fact that the virus can rapidly adapt to the presence of antiretroviral drugs, resulting in resistance to those drugs. To prevent or delay resistance development, clinicians therefore are using a regimen consisting of a combination of several types of antiretroviral drugs that target different stages in the lifecycle of the virus. Such regimens are called highly active antiretroviral therapy (HAART).<sup>[1]</sup> Drug types that are most commonly used in these combination regimens include: Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs) and Protease inhibitors (PIs). Most HAART regimens consist of at least three drugs from at least two different drug classes (two NRTIs and one NNRTI or two NRTIs and one PI).<sup>[2,3]</sup>

The introduction of HAART has led to a marked reduction in AIDS related morbidity and mortality. Since its introduction patients have started to live longer through dramatic decline in viral loads, sustained increase in CD4 counts and significantly increase life expectancy. However, co-morbid problems shown to be including optimal monitoring for treatment failure, the threat of emerging drug resistance, the availability of second line therapy and dyslipidemia.<sup>[4]</sup>

HAART associated dyslipidemia is characterized by increased levels of TGs, TC and LDL-C with low levels of HDL.<sup>[5,6]</sup> The metabolic abnormalities such as dyslipidemias, insulin resistance, hyperglycemia and changes in body fat distribution or lipodystrophy syndrome are classic examples of this situation. Long term exposure of HAART leads to dyslipidemias in the population increases the risk of cardiovascular diseases.<sup>[7,8]</sup>

Pathophysiological pathway for the clustering of metabolic disturbances observed in HIV infected patients on PI-based anti-retroviral (ARV) therapy [figure 1]. At cellular levels the initial signal is triggered by induction of endoplasmic reticulum stress which in turn inhibition of glucose uptake in adipocytes, insulin release by pancreatic  $\beta$ -cells and dysregulation of sterol regulatory element binding protein (SREBP) activity in the hepatocyte. As a consequence of alteration in protein expression, homeostasis in the oxidation of energy substrates is impaired with a shift toward increased lipolysis.

Ultimately, this represents a failure in fuel sensing and regulation that can further affect both lipid and glucose metabolism, triggering a vicious metabolic cycle.<sup>[9]</sup>

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. The use of herbal medicine has become more prevalent and the past few decades have witnessed a rapidly increasing demand worldwide.<sup>[10]</sup>

According to the WHO, more than 3.5 billion people in the developing world rely on medicinal plants as components of their healthcare. The vast majority of people (70-80%) in Africa consult Traditional Medical Practitioners (TMPs) for their healthcare. Traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary healthcare delivery not only in Africa but also in all countries of the world.<sup>[10]</sup>

Ethiopian plants have shown very effective medicinal value for some ailments of human and domestic animals thus medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs throughout the country.<sup>[11]</sup> The major reasons why medicinal plants are demanded in Ethiopia are due to culturally linked traditions, the trust the communities have in the medicinal values of traditional medicine and relatively low cost in using them.<sup>[12]</sup>

*Ocimum gratissimum* is herbaceous plant which belongs to the Lamiaceae family [Figure2]. It is one of the traditional medicinal plants which are widely used in various countries such as Nigeria, India, Brazil and including Ethiopia.<sup>[13]</sup> In Ethiopia, *Ocimum gratissimum* is Lamiaceae family and locally called Anchabi in Afan Oromo. Traditionally used for febrile illness. The leaf in fusion is smelled and the affected body part is massaged by the infusion during the bed times.<sup>[14]</sup> The plant is available in Ethiopia around some parts of regions where it is used for humans as traditional medicine. Therefore, this study is to investigate the antidyslipidemic properties of extract of *Ocimum gratissimum* leaves against HAART induced dyslipidemia in rats that have not been studied so far scientifically.

## METHODS

### Plant material collection and authentication

The fresh leaves of *Ocimum gratissimum* were collected from Eastern Wellega area of Nekemte town, 328 km west of Addis Ababa where the plant is densely distributed. After collection, the plant was authenticated by a taxonomist of Ethiopian National Herbarium (ETH) of Addis Ababa University, Ethiopia; voucher number 001/OG/2013 was given and deposited there for further reference.

### Preparation of plant extraction

The leaves of *Ocimum gratissimum* were rinsed with water to remove dirt and cleaned. This was followed reduced to small fragments, air dried under shade at room temperature and then powdered by using grinding mill. The coarse powdered leaves were weighed and 1400 g powder was macerated in 70% ethanol for 72 hours with mechanical shaking twice a day. This was repeated 3 times until the extract gave faint or no coloration. The extract was then filtered through Whatman filter paper No.1 and filtrate was evaporated to dryness under reduced pressure by rotavapor and further concentrated by water bath at 40°C. Then, gummy residue extract was packed in air tight brown glass bottles with proper label and kept in a refrigerator at 4°C until used for the preparation of stock solutions required in the subsequent experimental tests. From the above powder 98 g yield of gummy residue was obtained.

The percentage yield of extraction was calculated as follows.

$$\text{Percentage yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of powdered}} \times 100\%$$

$$\begin{aligned}\text{Percentage yield} &= \frac{98g}{1400g} \times 100\% \\ &= 7\% \text{ (w/w)}\end{aligned}$$

### Experimental animals

Thirty six albino wistar rats weighing 150-200g were obtained from Addis Ababa University, Pharmacology department for the study. Rats were given to acclimatization period in the animal house, School of Medicine, Addis Ababa University. Experimental animals were fed standard rat pellets and were allowed free access to water. The animals were maintained under standard laboratory conditions and were subjected to natural

photoperiod of 12 hours light: dark cycle. The experiment was performed after experimental protocol was approved by the Departmental Research and Ethics Review Committee (DRERC) by the protocol number 02/13. All rules applying to animal handling safety and care was observed as per the guidelines set by the national academies press, Washington, D.C., USA.

### **Extrapolate of HAART dose**

The doses of HAART were from humans and extrapolated to animals by the formula; Human Equivalent Dose (HED in mg/kg) = Animal Dose (mg/kg)  $\times$  (Animal Km  $\div$  Human Km), Where Km is a correction factor reflecting the relationship between body weight and body surface area.<sup>[15]</sup>

### **Animal grouping and drug dose**

Thirty six rats of same age and 150-200 g weight were randomly selected and divided into six groups containing each six. The dose of *Ocimum gratissimum* was selected on the basis of previous reports with some modifications.<sup>[16]</sup> The following dosing plan was adapted for the study.

Group I: Normal control received 1mL distilled water.

Group II: Stavudine + Lamivudine + Nevirapine (0.11 + 0.55 + 0.7mg/Kg) first phase and Lopinavir + Efavirenz (2.82 + 2.11mg/Kg) second phase respectively.

Group III: Stavudine + Lamivudine + Nevirapine (0.11 + 0.55 + 0.7mg/Kg) first phase and Lopinavir + Efavirenz (2.82 + 2.11mg/Kg) second phase respectively with 100 mg/Kg of OGE.

Group IV: Stavudine + Lamivudine + Nevirapine (0.11 + 0.55 + 0.7mg/Kg) first phase and Lopinavir + Efavirenz (2.82 + 2.11mg/Kg) second phase respectively with 200 mg/Kg of OGE.

Group V: Stavudine + Lamivudine + Nevirapine (0.11 + 0.55 + 0.7mg/Kg) first phase and Lopinavir + Efavirenz (2.82 + 2.11mg/Kg) second phase respectively with 300 mg/Kg of OGE.

Group VI: Stavudine + Lamivudine + Nevirapine (0.11 + 0.55 + 0.7mg/Kg) first phase and Lopinavir + Efavirenz (2.82 + 2.11mg/Kg) second phase respectively with 400 mg/Kg of OGE.

### Animals sample collection

After thirty days treatment the rats were fasted overnight (12 to 14 hours). Then, animals were anaesthetized and blood sample was collected by cardiac puncture using 5ml hypodermal syringe. Serum was separated after coagulated at room temperature for 30 minutes and centrifuged at 3000 rpm for 10 minutes. The obtained serum was stored at -20°C for biochemical studies.

### Serum lipid profile

Clinical chemistry laboratories offer many tests for lipid disorders. One of the most common tests is the lipid profile. This panel of tests includes measurements of TG, TC and cholesterol in the form of LDL-C and HDL-C were determined.

### Statistical analysis

The total variation and difference among means were analyzed through one-way analysis of variance (ANOVA). Post hoc Tukey's comparisons between the experimental and control groups were made using SPSS statistical software package Version 20.0. All data were expressed as mean  $\pm$  SEM. P value 0.05 and less was considered statistically significant.

## RESULTS

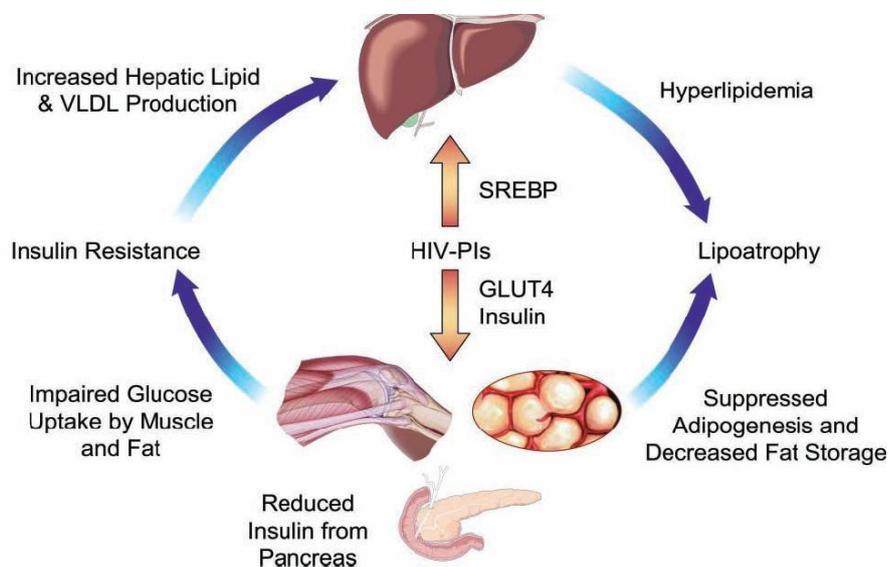
The plant was identified by a taxonomist of Ethiopian National Herbarium (ETH) of Addis Ababa University, Ethiopia and voucher number 001/OG/2013 given and deposited there for further reference.

### Effect of *Ocimum gratissimum* leaves extract on serum lipid profile in Highly Active

#### Antiretroviral Therapy administered albino wistar rats

The level of serum lipid profile of experimental albino wistar rats described in **figure 3, 4, 5** and **6**. Serum TC ( $78.20 \pm 3.80$  to  $104.8 \pm 5.89$  mg/dL) (**figure 3**), TG ( $83.20 \pm 14.14$  to  $182.40 \pm 20.60$  mg/dL) (**figure 4**) and LDL-C ( $21.56 \pm 5.83$  to  $62.14 \pm 7.25$  mg/dL) (**figure 6**) increased significantly ( $P < 0.05$ ) among HAART administered groups, group II rats as compared with the normal control rats. On the other hand, the level of HDL-C ( $40 \pm 4.8$  to  $6.18 \pm 1.08$  mg/dL) (**figure 5**) decreased significantly ( $P < 0.05$ ) in HAART administered group (II) rats as compared to the normal control.

Serum TC ( $104.8 \pm 5.89$  to  $78.39 \pm 4.66$  mg/dL) (**figure3**), TG ( $182.40 \pm 20.60$  to  $103.68 \pm 20.23$  mg/dL) (**4**) and LDL-C ( $62.14 \pm 7.25$  to  $19.03 \pm 1.29$  mg/dL) (**figure 6**) were significantly reduced whereas HDL-C ( $6.18 \pm 1.08$  to  $38.61 \pm 2.36$  mg/dL) (**figure 5**) was significantly increased with OGE treated groups; group VI as compared to HAART administered group (II) rats.

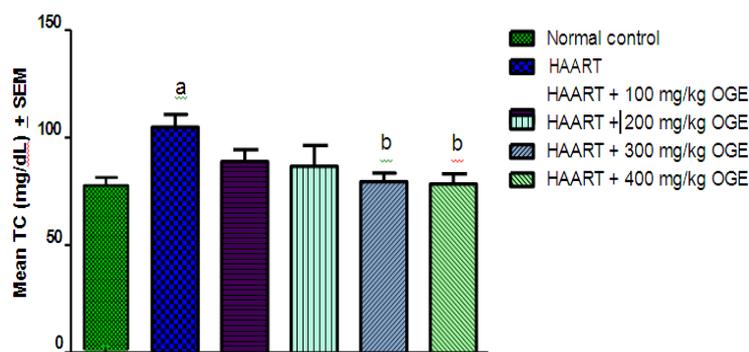


**SREBP**: Sterol regulatory element binding protein, **GLUT4**: Glucose transporter 4, **PIs**: Protease inhibitors

**Figure 1: Early direct effects of HIV protease inhibitors on intermediary metabolism (Flint et al., 2009).**

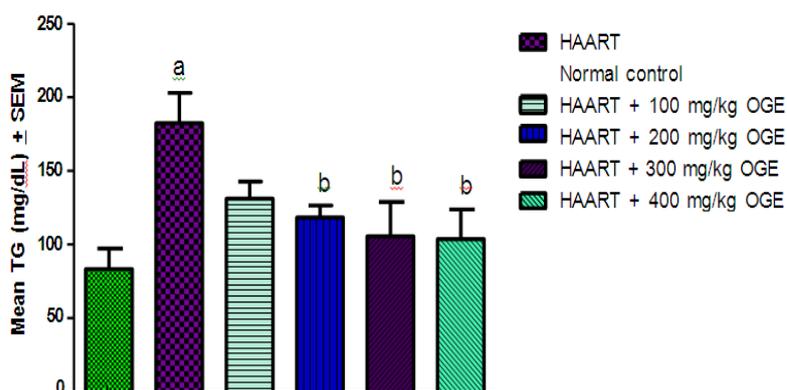


**Figure 2: Ocimum gratissimum, Anchabi, in Afan Oromo.**



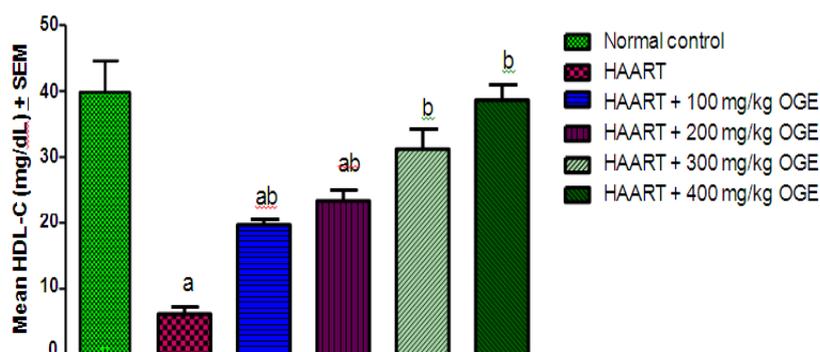
**Figure 3: Effect of Ocimum gratissimum on serum TC in HAART administered rats after 30 days.**

The values indicate mean  $\pm$  SEM (n=5). <sup>a</sup>p<0.05 compared with normal control values and <sup>b</sup>p<0.05 compared with HAART control values (One way ANOVA followed by Tukey test).



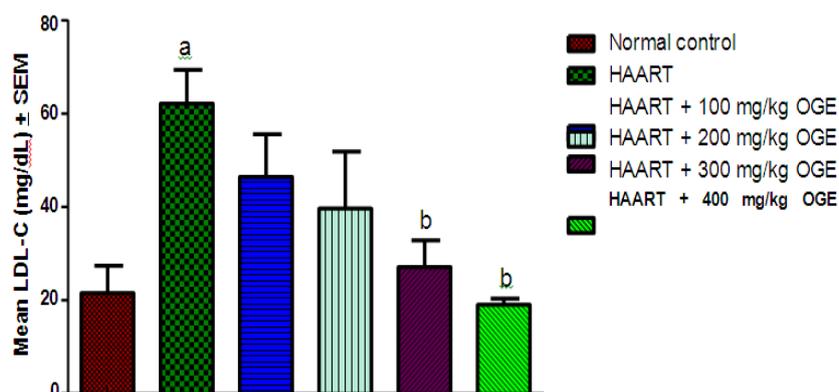
**Figure 4: Effect of Ocimum gratissimum on serum TG in HAART administered rats after 30 days.**

The values indicate mean  $\pm$  SEM (n=5). <sup>b</sup>p<0.05 compared with normal control values and <sup>b</sup>p<0.05 compared with HAART control values (One way ANOVA followed by Tukey test).



**Figure 5: Effect of Ocimum gratissimum on serum HDL-C in HAART administered rats after 30 days.**

The values indicate mean  $\pm$  SEM (n=5). <sup>a</sup>p<0.05 compared with normal control values and <sup>b</sup>p<0.05 compared with HAART control values (One way ANOVA followed by Tukey test).



**Figure 6: Effect of *Ocimum gratissimum* on serum LDL-C in HAART administered rats after 30 days.**

The values indicate mean  $\pm$  SEM (n=5). <sup>a</sup>p<0.05 compared with normal control values and <sup>b</sup>p<0.05 compared with HAART control values(One way ANOVA followed by Tukey test)

## DISCUSSION

Effective HAART for HIV infection has led to marked improvement in life expectancy for those infected with HIV. Despite reductions in the incidence of AIDS with effective treatment, patients continue to experience considerable morbidity and mortality from non AIDS illness such as premature cardiovascular disease, liver failure and renal failure.<sup>[17]</sup>

HAART leads to lipodystrophy is characterised by peripheral, subcutaneous lipotrophy and relative central fat accumulation. One or more of several metabolic abnormalities are typically associated with lipodystrophy: elevated TC, TGs, LDL-C and low levels of HDL-C.<sup>[18]</sup>

Antiretroviral drugs has been reported that ritonavir administered mice have increased of the active form of sterol regulatory element binding protein (SREBP)-1c in liver cells, which leads to an increased lipogenesis. In addition, it also slows down the intracellular degradation of APo- B-100, prompting an overproduction of VLDL particles. Finally, these drugs may inhibit the LDL receptor- related protein, reducing the clearance of VLDL from circulation.<sup>[19]</sup> Lipotrophy caused by nucleoside analogues has been proposed to result from inhibition of mitochondrial DNA polymerase gamma within subcutaneous adipocytes. An alternative hypothesis is intracellular depletion of pyrimidine precursors, rather than depletion of mitochondrial DNA. Dideoxynucleosides have a high potential for mitochondrial toxicity, which causes defective beta-oxidation of free fatty acids.<sup>[20]</sup>

The investigated effects of HAART on mice reported that these drugs were caused a dyslipidemia which contributes to the development of cardiovascular diseases. The present investigation is in line with this observation, HAART administration resulted in elevated levels of TC, TG and LDL-C and drastic decreased HDL-C as compared to normal control.<sup>[21]</sup>

Oral administration of various doses of hydroethanolic OGE to HAART intoxicated rats resulted in gradually restored the affected serum lipid profile near to the control value (Figure 3, 4, 5, 6). This evidently suggested that antidyslipidemic effect of the extract has been improving the functional integrity of the metabolism and normalizing the serum lipid profile. This antidyslipidemic effect of *Ocimum gratissimum* could be related to its chemical composition, which shows the presence of alkaloids, flavonoids, saponin and cardiac glycosides.<sup>[22]</sup> All these components are known to reduce serum lipid level in animals. Especially, saponins lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase in its fecal excretion.<sup>[23]</sup> Hence, the present study implies the antidyslipidemic activities of *Ocimum gratissimum* in HAART induced dyslipidemia of rats.

## CONCLUSION

The present study has made an attempt to study antidyslipidemic activities of a widely available plant *Ocimum gratissimum* which is in use for different ailments but which is not scientifically proved against HAART associated dyslipidemia until now. Based up on the present study, Oral administration of hydroethanolic extract of *Ocimum gratissimum* leaves have significant reduction of serum lipid profile such as total cholesterol, triacylglycerol, low density cholesterol and increased high density cholesterol of rats with highly active antiretroviral therapy administered groups of rats.

From all these findings, it can be concluded that the plant *Ocimum gratissimum* has significant antidyslipidemic role as evidenced by biochemical parameters. The present findings also provide scientific evidence of *Ocimum gratissimum* in treating dyslipidemia, oxidative stress occurred due to taking of highly active antiretroviral therapy for treatment of HIV/AIDS.

## COMPETING INTERESTS

The authors declare that they have no financial or personnel competing interests.

### **AUTHORS' CONTRIBUTIONS**

Abdisa T, performed almost all of the experiments and analyzed the data. Daniel S, supervised part of the study. Melaku U, designed the research, supervised and reviewed the manuscript. Gnanasekaran N, critical evaluation of the experiment and devoted his time on reading part. Yididya B, interpretation of results and editing of the paper. All authors read and approved the final manuscript.

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