Salubrious effects of Calpurnia aurea seed extract on HAART Hepatotoxicity

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ABSTRACT

Aim: The effect of hydroethonolic seed extract of *Calpurnia aurea* was evaluated against HAART induced free radical reactions in liver and liver cell damage in Wistar male albino rats.

Background: Highly active antiretroviral therapy (HAART)-correlated hepatotoxicity make difficult the management of patients infected with human immunodeficiency virus (HIV), raise medical costs, changes the prescription prototypes, and affects the principle recommendations.

Materials and Methods: Matured dried seeds of *Calpurnia aurea* were collected, powdered and extracted using 70% ethanol. Preliminary phytochemical screening and *in-vitro* antioxidant properties of the extract were carried out. Thirty rats of same age and 150-200g weight were selected and divided into five groups containing six each. The HAART and different doses of the *Calpurnia aurea* seeds extract (100, 200 and 300 mg/kg) administered orally for 35 days. The end of the experiment day the rats were fasted overnight. Then blood samples were collected by cardiac puncture for biochemical studies after sacrificed by cervical dislocation and liver was excised from the rats for histopathological studies. The hepatoprotective effects of the seeds extract against HAART liver toxicity in rats were evaluated by monitoring the levels of alkaline phosphatase (ALP), amino transferases (AST, ALT), and histopathological analysis. In addition, the antioxidant properties of the seed extract against HAART induced alteration in rats liver antioxidant profile were evaluated by monitoring the levels of SOD, CAT, GHS, MAD and TAC analysis.

Results: Increased free radical reactions, ALP, amino transferases release and decrease antioxidant profile were detected in HAART treated rats. The rats treated with the extract (300 mg/kg) reduce the HAART induced liver toxicity but minimum dose of extract (100 mg/kg) did not show any significant change against HAART altered parameters.

Conclusion: This study suggests that the *Calpurnia aurea* seed extract have hepatoprotective potential, thereby justifying their ethnopharmacological uses.

Keywords: Hepatotoxicity, HAART, *Calpurnia aurea*, antioxidant, amino transferases.
1. INTRODUCTION

The arrival of highly active antiretroviral therapy (HAART) in the management of human immunodeficiency virus (HIV) infection has significantly reduced the incidence of opportunistic infection as well as improved morbidity and mortality among HIV patients. However, along with these positive outcomes, HAART is associated with a host of unpleasant reactions such as hepatotoxicity, hyperlipidemia, hyperglycemia, and lactic acidosis. Hepatotoxicity can interrupt HIV therapy and cause an increase in morbidity and mortality [1]. Adverse effects have been reported to be associated with different classes of anti-HIV drugs, including nucleoside reverse transcriptase inhibitors, non-nucleoside analogue reverse transcriptase inhibitors, and HIV protease inhibitors [2]. Aside from host factors, several individual antiretrovirals or classes have been independently associated with hepatotoxicity, such as nevirapine, protease inhibitors, high doses of ritonavir (600 mg/day), and prolonged zidovudine or stavudine exposure. [3-5] Nucleos(t)ide-reverse transcriptase inhibitors (NRTIs) has their ability to hinder Pol-γ in mitochondrial, the DNA polymerase is an accountable for the synthesis of mitochondrial DNA. In addition, other groups of anti-HIV drugs such as non-nucleoside reverse transcriptase inhibitors and protease inhibitors interfere with mitochondrial toxicity [6]. The mechanisms of drug-induced liver damage are not forever known, but when they are investigated mitochondrial dysfunction is frequently there [7–9]. In addition, innate and adaptive immune responses are additional factors of notice which conclude the sequence and harshness of liver damage [10, 11]. Detailed review mechanisms of drugs induced liver injuries focusing on pathogenesis are available elsewhere. [12-15]

HAART hepatotoxicity confuse the management of HIV infected patients, raises the medical costs, alters the prescription patterns, and has an impact on official treatment recommendations. Several mechanisms of liver toxicity in patients receiving HAART had been recognized. Although rare, HAART-related liver damage may have shocking consequences. Among clinical syndromes of HAART liver toxicity, allergic reactions and lactic acidosis are recognized. Among the latter, HAART-related liver fibrosis, NASH (Nonalcoholic Steatohepatitis), nodular regenerative hyperplasia, and portal hypertension are high risk. Prevention is the best plan to reduce the luggage of hepatotoxicity and includes appreciation of antiretrovirals’ liver safety profile and of vulnerable hosts. Management of hepatotoxic trial includes discontinuation of alleged causes and modifies in HAART regimens [16].
Medicinal plants play an important role in the lives of rural people particularly in remote parts of developing countries with few health facilities. The plants, fruits, and compounds described could offer novel alternatives to the limited therapeutic options that exist for the treatment of liver diseases. Hepatoprotective activity of phytochemicals, were related to their antioxidant potential [17, 18]. *Silybum marianum* commonly known as ‘milk thistle’; (family of Asteraceae) seeds contain flavonoid such as silymarin is recognized as a hepato-protective agent of herbal origins. It also has clinical applications in the treatment of liver related disorder such as toxic hepatitis, fatty liver, cirrhosis, ischemic injury, and viral hepatitis via its anti-oxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory and liver regenerating effects, [19, 20, and 21].

*Calpurnia aurea* is a genus of FLOWERING PLANTS within the family of *fabaceae*. Literature survey brings to light that, the leaf and stem of *C. aurea* has been used for different human and animal disease [22]. Mulata *et al.*, reported that the 70% ethonolic extract of *C. aurea* seed revealed the presence of tannins, flavonoids, terpenoids, saponins, steroids, glycosides, alkaloids but absent anthraquinone, yet seed containing more tannins and alkaloids than the leaves. This extract is an effective counter measure for the toxic haematopoietic effects of HAART [23, 24]. The aim at the present study was to explore protective roles of hydroethanolic extract of *C. aurea* seed in HAART -induced hepatotoxicity.

4. MATERIALS AND METHODS

Plant materials

The *C. aurea* plant with flower and seeds were collected from south Gondar, northern Ethiopia in June 2013. The plant has identified and authenticated by taxonomist of Ethiopian National Herbarium of Addis Ababa University and its voucher number is 001/2006. The seeds were washed thoroughly 2-3 times with running tap water, and dried in shade and reduced to a fine powder.

Extraction and Assay of *In-vitro* Antioxidant Activity by Spectrophotometric Method

The powdered seeds were weighed 100 g and macerated in 70% ethanol for 72 hours with mechanical shaking and it was filtered through Whatman No.1 filter paper. Then filtrate was evaporated using rotary evaporator and dried at 40°C. The yield was found to be 17.62 % w/v. Preliminary phytochemical tests
were performed by standard phytochemical test procedures. Assay of \textit{in-vitro} antioxidant activity by DPPH radical-scavenging activity of the extract was examined as previously described [25].

**Acute Oral Toxicity Test**

The acute oral toxicity test indicated that no visible signs of acute toxicity and mortality were observed at the dose of 300 mg/kg body weight.

**Animals**

Thirty adult apparently 12 weeks’ old healthy male albino rats of weighing about 140 – 200 g were used in the present study and housed in polypropylene cages and maintained standard laboratory conation. They were provided with standard pellet rat diet supplied by Kality Animal Nutrition Production Ltd., Addis Ababa Ethiopia, and water \textit{ad libitum}. The research protocol was approved by the Research & Ethics Review Committee (DRERC) of the department of medical Biochemistry, Addis Ababa University with approval number SOM/BCHM/012/2013 EC. All the animal experiments were carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

**Extrapolation of HAART Dose**

The humans doses of HAART drug were extrapolated to animals by the formula; Human Equivalent Dose (HED in mg/kg) = Animal Dose (mg/kg) × (Animal Km ÷ Human Km), Where Km is a correction factor reflecting the relationship between body weight and body surface area. [26]. The table I shows the average Km value of most frequently used laboratory animals and human adults.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>3</td>
</tr>
<tr>
<td>Rat</td>
<td>6</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>8</td>
</tr>
<tr>
<td>Rabbit</td>
<td>12</td>
</tr>
<tr>
<td>Dog</td>
<td>20</td>
</tr>
<tr>
<td>Human Adult</td>
<td>37</td>
</tr>
</tbody>
</table>

Table I: Average Km values of laboratory animals and human adult
Based on the above data the dosages of regimen were given for group II to V as follows Stavudine+ Lamivudine+ Nevirapine (0.11 + 0.53 + 0.7) mg/Kg administered by for 35 days.

**Animal grouping and drug dose**

Group- I normal control, given distilled water only  
Group- II positive control, given HAART drugs only  
Group- III HAART drugs + 100 mg/kg of CASE (CASE: *Calpurnia aurea* Seed Extract)  
Group- IV HAART drugs + 200 mg/kg of CASE  
Group- V HAART drugs + 300 mg/kg of CASE

**Blood Sample Collection and analysis**

The end of the experiment day the rats were fasted overnight, sacrificed by cervical dislocation and blood has been collected by cardiac puncture and serum was obtained. The serum was stored at -20° C for biochemical studies.

**Serum enzyme assay**

The appropriate kits were used for the determination of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to Reitman and Frankel [27], alkaline phosphatase (ALP) according to Belfield and Goldberg [28]. The enzyme activity was expressed as units/liter computed directly from the absorbance values. Serum total protein was measured according to (Gornall *et al*., [29], and Albumin according to Doumas *et al*., [30]. Total and direct bilirubins were determined according to Walters and Gearde, 1970 [31].

**Liver antioxidant**

Liver was homogenized (10 % w/v) in ice–cold 0.1 M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 3,000 rpm for 15 min at 4° C and the resultant supernatant was used for assessing different oxidative stress markers. Superoxide dismutase (SOD) was determined according to Nishikimi *et al*., [32], catalase (CAT) according to Aebi, [33], reduced glutathione (GSH) according to Beutler *et al*., [34], malondialdehyde (MDA) according to Mihara and Uchiyama[35], total antioxidant capacity(TAC)
according to Koracevic et al., [36] and Thiobarbituric Acid Reactive Substances (TBARS) according to Yagi [37].

Liver histopathological studies

Liver sections taken immediately from the liver, fixed in 10 % buffered formalin, dehydrated in ethanol (50–100 %), cleared in xylene, and embedded in paraffin. Sections (4–5 lm thick) were prepared and then stained with hematoxylin and eosin (H–E). The sections were examined for the pathological findings of hepatic changes.

Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between the groups were tested using one-way ANOVA followed by Dennett's post-hoc test. A P less than 0.5 were considered significant

2. Results

Phytochemicals and invitro antioxidant activity

The preliminary phytochemical analysis of 70% ethanolic extracts from the C.aurea seeds showed the presence of tannins, flavonoids, terpenoids, saponins, steroids, glycosides, alkaloids compounds. The extract showed significant activities in all antioxidant assays compared to the reference antioxidant ascorbic acid in a dose dependent manner. In DPPH, scavenging assay the IC$_{50}$ value of the extract found to be 58.26µg/ml while the IC$_{50}$ value of the reference standard ascorbic acid was 52.92 µg/ml.

Improvement of liver functions in CASE treated rats

The activities of aminotransferase (AST and ALT), ALP levels as well as serum TP, albumin, TB and DB in the control, HAART, and different doses of CASE-HAART administered rats are presented in Table 2. Administration of HAART -induced significant increase (P≤0.001) in serum AST, ALT, ALP TB , DB as well as significant decrease in the serum TP and albumin content as compared to control. Treatment for different doses of CASE especially the group 5 rats (300mg/kg CASE) significantly (P≤0.01) decrease serum ALT, AST,ALP ,TB and DB and significant increase in the serum TP and albumin content near to the control . The results showed that administration of the CASE shows a significant positive change in the liver function markers of HAART administered rats.
Table 2. The influences of the different dose of CASE on the levels of serum ALT, AST, ALP, Total Protein, Albumin, Total and Direct Bilirubin in HAART administered rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>ALP U/L</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Direct bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.02 ±3.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.72 ±3.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.14 ±6.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.71±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.70 ±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HAART</td>
<td>48.48 ±10.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.43 ±4.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>131.40 ±10.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.32±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.31 ±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.56 ±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HAART+100 mg CASE</td>
<td>45.60 ±14.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.36 ±4.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>129.24 ±8.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.52±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.36 ±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.52 ±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 ±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HAART+200 mg CASE</td>
<td>37.00 ±2.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.56 ±4.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>103.33 ±8.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.93±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.48 ±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.45 ±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.13 ±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HAART+300 mg CASE</td>
<td>31.80 ±2.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.62 ±5.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.34 ±4.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.32±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.57 ±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43 ±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.12 ±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Antioxidative activity of CASE in the livers of HAART administered rats

Oxidative stress markers such as superoxide dismutase (SOD) catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA) levels and TBARS as well as total antioxidant capacity (TAC) in liver tissue of control, HAART and different doses of CASE-HAART administered rats in Table 3. Data showed that
HAART administration caused a significant decrease (P≤0.001) in SOD and CAT activities, GSH level, TAC and significant increase (P≤0.001) of TBARS as compared to control. Treatment of rats with the CASE (200,300 mg/kg body weight) significantly increased (P≤0.001) the level of SOD, CAT, GSH and TAC as compared to HAART treated group. **HAART treatment significantly (P≤0.001)** increased the level of MDA in the liver tissue as compared to control, MDA levels were assessed as an indicator of lipid peroxidation. Pretreatment with CASE (100, 200 and 300 mg/kg) had more or less prevented this trend, according to the amount of CASE (p < 0.05). When the dose reached 300 mg/kg, the results were as good as compared to the dose of 100 mg/kg.

**Table 3** Effect of the different dose of CASE on the levels of SOD, CAT, GSH, MDA, TBARS, TAC, in liver homogenate of HAART administered rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD U/g</th>
<th>CAT U/g</th>
<th>GSH mg/g</th>
<th>MDA nmol/g</th>
<th>TBARS</th>
<th>TAC µmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>±16.17 ± 0.73a</td>
<td>±1.84 ± 0.02a</td>
<td>±0.73</td>
<td>±44.86 ± 0.98a</td>
<td>±374.93 ± 13.01a</td>
<td>±8.32 ± 1.31a</td>
</tr>
<tr>
<td>HAART</td>
<td>±7.22 ± 0.41b</td>
<td>±0.70 ± 0.035b</td>
<td>±0.76c</td>
<td>±24.39 ± 0.76c</td>
<td>±835.06 ± 19.14b</td>
<td>±15.42± 2.12b</td>
</tr>
<tr>
<td>HAART+100 mg CASE</td>
<td>±10.86 ± 0.74c</td>
<td>±1.51 ± 0.033c</td>
<td>±0.033c</td>
<td>±29.17 ± 1.59d</td>
<td>±482.44 ± 12.74c</td>
<td>±14.32± 2.32b</td>
</tr>
<tr>
<td>HAART+200 mg CASE</td>
<td>±12.99 ± 0.43d</td>
<td>±1.65 ± 0.027d</td>
<td>±0.027d</td>
<td>±38.64 ± 1.13c</td>
<td>±458.79 ± 10.46c</td>
<td>±10.23± 1.21c</td>
</tr>
<tr>
<td>HAART+300 mg CASE</td>
<td>±14.04 ± 0.49d</td>
<td>±1.75 ± 0.038a</td>
<td>±0.038a</td>
<td>±40.41 ± 1.41b</td>
<td>±451.09 ± 9.12c</td>
<td>±9.23± 1.72ac</td>
</tr>
</tbody>
</table>

**Histopatological observations**
Histopathology of the normal control rat liver (Fig. 1) shows preserved cytoplasm, no vacuolation, no lymphocyte infiltration (inflammation), and no area of necrosis.

However, histopathology of HAART received rat liver (Fig. 2), shows chronic inflammation, necrosis, lymphocyte infiltration (inflammation), sinusoidal dilation and cytoplasmic vacuolation.
Histopathology of group – III rat liver (Fig. 3), shows focal lymphocyte infiltration (inflammation), and sinusoidal dilation.

Histopathology of group– IV rat received 200mg/kg CASE + HAART (Fig. 4), shows sinusoidal dilation and cytoplasmic vaculation but lower than group-II and Group – III rats.
Histopathology of group– V rats received 300mg/kg CASE + HAART (Fig. 5), depicts generally no abnormal features and the cords of hepatocytes were distinct, sinusoids were well demarcated, no vacuolation, no lymphocyte infiltration (inflammation), no significant area of inflammation and necrosis.

3. Discussion

The administration of HAART to the rats resulted in marked elevation of serum enzymes ALT, AST, ALP and bilirubin. Membrane disintegration of hepatocytes with subsequent release of AST, ALT and ALP, among others, is one of the consequences of HAART -induced lipid peroxidation [38]. Among the liver specific enzymes, mostly alanine aminotransferase considered very responsive for pointers of hepatotoxic as well as hepatoprotective or curative effects of different compounds. The activities of these enzymes are used to assess the functional status of the liver and as the biochemical markers of liver damage [39]. Both AST and ALT levels rises in the plasma due to toxic compounds that promotes the liver cell necrosis. Decreased levels of transaminases indicate reduces the hepatocytes necrosis and safety of hepatocytes against damage caused by hepatotoxin. Normal liver functions are typify by the balanced activities of
serum marker enzymes AST, ALT, ALP and bilirubin as well as albumin level [40]. Liver toxicity caused by antiretroviral therapy can be inflicted through several mechanisms. The pathogenesis often remains enigmatic. Five categories are proposed: direct mitochondrial inhibition, direct cell stress, hypersensitivity reactions, immune reconstitution in the presence of viral hepatitis co-infection, and disturbances of lipid/sugar metabolism and steatosis [41]. Some antiretrovirals or classes may be toxic for the liver through different pathways, a feature which is characteristic of drug-induced hepatotoxicity in general[42]. All or several members in three antiretroviral classes can cause disturbances in lipid and sugar metabolism, which seem to be contributors to a not well-defined steatohepatitis syndrome. nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) [31]. Mitochondrial liver toxicity leading to steatosis and lactic acidosis, which is secondary to mitochondrial RNA depletion by NRTI use, is particular to that class [43]. Hypersensitivity reactions with liver involvement are common to NNRTIs but are possible also for specific drugs in other classes [44-50].

Since phenolic and flavonoid compounds are documented as the bases of the antioxidant activity of plant extracts, we determined the phenolic and flavonoid content of CASE. Several studies have demonstrated the protective effects of herbals against experimentally induced liver injury. In addition, a number of herbals show promising activity including silymarin against liver cirrhosis. Silymarin, a reference drug, is a combination of flavonoids and polyphenols. Silymarin has membrane-stabilizing and antioxidant behaviors, it endorses hepatocyte regeneration, decreases inflammatory reactions, and inhibits fibrogenesis [51]. Therefore, the CASE was chosen for evaluation of the hepatoprotective activity in HAART induced hepatotoxicity in a rat model and it was found to have good hepatoprotective effects evidenced by suppression of HAART-induced oxidative stress in the liver of rats, and attenuating the morphological changes caused by HAART. Moreover, the correlation between antioxidant and hepatoprotective activity was also examined. The results suggested that the possible mechanism of this activity might be due to free radical-scavenging and antioxidant activity of the CASE.

**HAART may induce (i) oxidative stress (ii) decrease in free radical scavenging protection, or (iii) a failure to restore oxidative damage. Hapatotxins are initially damage the centrilobular region of liver where they are high levels of cytochrome P450 oxidases that mediated their conversion to toxic electrohilic metabolites. These toxic metabolites can covalently bind to proteins lipids, DNA forms adducts followed**
by reactive oxygen species production (ROS), lipid peroxidation, relases of pro-inflammatory cytokines, glutathion depletion and cell death [52]. Therefore, free radical-scavenging is the most significant way to protect the liver against hepatotoxicity induced by HAART. In the present study in the CASE, tannins and flavonoid have been identified. Tannins are strong secondary antioxidant have ability to chelate metal ions such as Fe$^{2+}$, not like primary antioxidants like donate hydrogen atom or electron. [53]. The prevention of membrane lipid peroxidation by tannin can act via the inhibition of cyclooxygenase [54, 55]. Flavonoids are able to inhibit D-galactosamine and CCl$_4$-induced hepatotoxicity in experimental models due to their potent anti-oxidant or free radical scavenging properties [56]. This active principle may account for the pharmacological properties of CASE.

We observed that decreases in glutathione (GSH) level, albumin and increase in oxidative stress in rats those who are received HAART. Glutathione well known reducing substrate important role as defense mechanism in oxidative damage. In GSH redox cycle, glutathione, act as a direct endogenous scavenger of hydroxyl radicals, involved in detoxification of toxic substances in the liver [57]. HAART reduces GSH synthesis, increased GSH use, or limited intracellular decrease of its oxidized form (GSSG) [58]. In liver GSH deficiency may be impaired in reducing ability, immune function, protein biosynthesis, accumulations of lipid peroxidation products and detoxification capacity. Reduced detoxification ability in the liver may lead to the accumulation of toxic metabolites in liver cell leading to liver damage [59]. In the present study, the activities of hepatic GSH and antioxidant enzyme in HAART-treated rats were markedly weakened. The administration of CASE possesses potent hepatoprotective activity in vivo, which might be due to restoration of the GSH level and therefore the amount of glutathione available in the cell for HAART detoxification. In acute silymarin treatment in mice to increase the hepatic GHS, via direct effect on the metabolism of sulfur containing amino acid in liver cell. Silymarin increased the amount of metabolites generated from homocysteine in the transsulfuration pathway (cystathionine, cysteine, and glutathione), elevated the activity of cystathionine β-synthase, while down-regulated cysteine dioxygenase. It concluded that Silymarin enhances hepatic glutathione generation by elevating cysteine availability via increment in cystein synthesis and an inhibiting of its catabolism to taurine [60]. Moreover Gnanasekaran et al., reported that pretreated with an aqueous leaf extract of Tridax procumbens in cultured mouse hepatocytes had significantly higher hepatocellular GSH levels [61]. This active principle may account for the pharmacological properties of CASE.
The salubrious effects of CASE against HAART-induced liver injury in rats may be due to its antioxidative properties. Further safety and efficacy studies are need to elucidate its mechanism of action in detail that CASE itself reacts with the reactive oxygen species or boosting the antioxidant enzyme and GSH production.

ACKNOWLEDGEMENT
Mr. Feysa Chala from EHNRI chemistry laboratories and Mr. Kissi Mudi from phytochemistry laboratory, Mr. Yohanis G. and Mohamed M. from biochemistry laboratory, Aster Seyoum and Mr. Tesfay Getachew from the animal laboratory for their kind assistance during laboratory works.

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