Hepatoprotective and antioxidant activity of methanolic leaves extract of *Cassia arereh* in CCl₄-induced rat liver damage

Abbas AY, Muhammad FI, Dallatu MK, Abubakar AL and Sahabi SM

Abstract
Liver disease is still a worldwide problem. *Cassia arereh* is one out of many medicinal plants in Northern Nigeria used for the management of liver diseases. The study was designed to evaluate the hepatoprotective effects of *Cassia arereh* against CCl₄-induced liver damage in rats. Standard analytical methods were employed in all the parameters assessed. Administration of methanolic leaves extract of *C. arereh* at doses of 50, 100, 150, 200 mg/kg body weight significantly (P<0.05) reduced the elevated serum liver marker enzymes and lipid peroxidation index (Malondialdehyde) and also increased the levels of total protein, albumin and endogenous antioxidants when compared to CCl₄ - induced not treated group. The hepatoprotective effect observed might be due to its rich phytochemical constituents.

Keywords: Hepatoprotective, antioxidant, *cassia arereh*, Phytochemical, histopathological, Silymarin

1. Introduction
Liver is one of the most important organs in human body. It is involved in regulation of various biochemical and metabolic functions and is involved in synthesis of various substances in the body [1]. The liver takes up glucose, minerals, and vitamins from portal and systemic blood and stores them. The hepatocytes also regulate blood levels of substances such as cholesterol and glucose and also helps maintain general body homeostasis [1]. The liver is continuously and variably exposed to environmental toxins, drugs and alcohol, viruses etc and these can eventually lead to various liver disease like hepatitis, cirrhosis and other liver impairments [2]. Antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by neutralizing free radicals [3]. Although, almost all organisms possess antioxidant defense and repair systems but these systems can be over whelm resulting in oxidative stress that lead to tissue damage. Antioxidant defense system comprises enzymatic (Catalase, Superoxide dismutase, glutathione peroxidase etc) and nonenzymatic (Vitamin E, C and β-carotene) that trap and destroy free radicals [4].

Liver disease is still a major threat to public health [5]. Modern medicines are increasingly complemented with medicinal plants for the management of liver diseases [6, 7]. This usage of herbal medicine by a large proportion of the population in the developing countries is largely due to high cost of synthetic drugs, expensive health care and adverse side effects [6]. Plants have been used by man as source of food, medicine, shelter, clothing, cosmetics, flavours, and spices since the creation of mankind [8, 9]. The use of medicinal plants by man is probably as old as the duration of human settlement on earth [10]. Medicinal plants contain phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins and sterols, generally known to be part of bioactive components in any ethno-medical plants [11]. Flavonoid and other phenolic compound of plant origin play roles of scavenging activity and inhibition of lipid peroxidation [7]. In traditional systems of medicine, plants were claimed to be effective and used successfully to alleviate multiple liver disorder [12]. Many plants and plant products are recommended for the treatment of liver diseases such as, *Moringa Oleifera*, *Ocimum gratissimum*, *Solanum nigrum*, *Balanties aegyptiaca*, *Khaya senegalisens* [13] (Baytop, 1999), and most times are found to offer significant relief [14].

*Cassia arereh* is a small tree which belongs to the family Caesalpiniaeae. It is found in the Sudan savannah, on shallow but quite rich soil [15]. It is locally called Malga, Maleduwa, Mihuski or Dandarazo in Hausa; Cabbi or Jutihi in Fulfulde; Mihuski in Gwari; kurnggilang in Cameroun, Ethopia, and Eritrea. Almost all parts of the plant are used locally as medicine [16].

*Correspondence*  
Abbas AY  
Department of Biochemistry,  
Faculty of Science, Usmanu Dan fodiyo University P.M.B. 2346,  
Sokoto, Nigeria
The leaves of the plant was reported to have antioxidant activity and antihaemolytic activity [17]. In view of the fact that there are little information regarding the use of the leaves extract of *C. arereh* as hepatoprotective agent, this study was designed to evaluate the hepatoprotective effect of the leaves extract of *C. arereh*.

2. Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents used were of analytical grades. They were all purchase from BDH Chemicals, UK, Sigma-Aldrich, UK, Thermo Fisher Scientific, Nigeria, Randox Laboratories, UK and Cayman Chemicals, USA.

2.2 Plant Materials

Fresh sample of the leaves of *Cassia arereh* was collected from Zuru, Kebbi State, Nigeria. The sample was taken to the Herbarium of the Botany unit, Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria, where it was identified and authenticated by a plant taxonomist Mal. Abdulazeez Salihu. A Voucher specimen was submitted to the Herbarium for future reference and voucher specimen number was obtained (UDUH/ANS/0113). The identified leaves of *Cassia arereh* was carefully detached from the stalks, washed with distilled water (to remove sand particles) and air dried (away from sun, dust and intense heat) under the shade in the laboratory. The leaves using a wooden mortar and pestle were reduced into small pieces, was weighed and stored in a specimen bottle until required for use.

2.3 Experimental Animals

Sixty (60) albino rats(Wistar strain) weighing 120-150g of both sexes were obtained from animal house, Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept in a well ventilated room under supervision in the animal house with free access to food and water *ad libitum*. They were kept for two weeks to acclimatize.

2.4 Methods

2.5 Preparation of Plant Extracts

Small pieces of leaves (200g) were extracted with two litres of methanol at room temperature overnight and were filtered with Whatman No. 1 filter paper. The filtrate was concentrated to dryness using rotary evaporator at 45°C and with Whatman No. 1 filter paper. The filtrate was left overnight and were filtered from the stalks, washed with distilled water (to remove sand particles) and air dried (away from sun, dust and intense heat) under the shade in the laboratory. The leaves using a wooden mortar and pestle were reduced into small pieces, was weighed and stored in a specimen bottle until required for use.

2.6 Phytochemical Analysis

Volatile oils, tannins, were estimated by the method of Harborne [18], terpenes and steroids were estimated by the method of Sofowora [19], resins, alkaloids, cyanogenic glycosides, anthraquinone glycosides, cardiac glycosides were estimated by the method of Trease and Evans [20], glycosides, saponins were estimated by the method of El-Olemyl *et al.* [21], flavonoids was estimated by the method of Boham and Kocijap [22].

2.7 acute toxicity studies (Determination of LD50)

After acclimatization period, the acute oral toxicity study was carried out according to organization for economic and cultural development (OECD) method [23]. Five (5) randomly selected animals were used. For limit test dose, 5000 mg/Kg body weight (bw) of the extract was given in a single dose. Each animal was dosed and observed one after the other. Observation for the first 8hrs, 14hrs, 24hrs, 48hrs and then up to 14 days for signs of toxicity like tremors, itching, depression, weakness, food and water refusal, salivation and death if any, was recorded. If three (3) or more animals died within 48hrs, the LD50 is less than 5000mg/kg and if one (1), two (2) or none animal died within 48hrs, the LD50 is greater than 5000mg/kg.

2.8 Experimental Design

Induction of hepatotoxicity was done with slight modifications according to the method of Guntupalli *et al.* [24]. The rats were divided into seven groups, of six rats each. **Group I:** (control) animals were administered a single daily dose of liquid paraffin (1ml/kg body weight, p.o.) for fourteen days. **Group II:** (Induction control) received 30 % carbon tetrachloride (1ml/kg body weight, i.p.) in liquid paraffin for every 72hrs for fourteen days. **Group III IV V VI:** received the methanol leaves extract of *Cassia arereh* (50, 100, 150 and 200mg/kg), respectively once a day for fourteen days and followed by CCl4 induction for every 72hrs. **Group VII:** received silymarin, a known hepatoprotective compound (Sigma-Aldrich, UK), at a dose of 100 mg/kg, p.o., once a day for fourteen days and followed by CCl4 induction for every 72 hrs. The animals were sacrificed after 48 hrs of CCl4 induction under chloroform anesthesia, blood and liver samples were collected. The blood was allowed to clot and the serum was separated by centrifuging at 3000rpm for 5 minutes. The serum was collected using Pasteur pipette into the sample bottle. The serum was used for biochemical estimations (GGT, AST, ALT, ALP, Total protein, Albumin, Bilirubin), vitamin C, vitamin E, catalase, superoxide dismutase, glutathione peroxidase MDA, reduced GSH. Some part of the liver sample was perfused with cold 0.86% KCl homogenized and centrifuged at 9000g for 20 minutes to obtain post mitochondrial supernatant for the estimation of enzymatic and non-enzymatic antioxidants. The other part of the liver was placed in 10% formalin for histopathological studies.

2.9 Biochemical Analysis

Serum Alanine Aminotransferase and Serum Aspartate Aminotransferase (AST) activities were ascertain using the method of Reitman and Frankel [25] (Assay kit: Randox laboratories, UK). Total protein in the blood was determined by Biuret method of Gomall [30]. AST, ALP, Total protein, Albumin, Bilirubin were estimated using the method of Bochem [31]. Gamma-Glutamyl Transferase Activity Assay was employed. Gamma-Glutamyl Transferase Activity Assay (GGT) by the method of Szasz and Bergmeyer [30] (Assay kit: Randox laboratories, UK).

2.10 Assessment of Antioxidant Activity

The liver was perfused with 0.86 % cold saline to completely remove the red blood cells. It was suspended in 10 % (w/v) ice-cold 0.1m phosphate buffer (pH 7.4). The liver was cut into small pieces, weighed and homogenized. The
homogenate was used for the estimation of enzymatic and non-enzymatic antioxidants.

Estimation of serum vitamin C by the method of Rutkowski et al. [31]. Estimation of serum vitamin E by the method of Rutkowski et al., [32]. Catalase by the method of Beers and Sizer [33]. (Assay kit; Cayman Chemical, USA). Reduced glutathione (GSH) by the method of Patterson and Lazarow [34], (Assay kit; Cayman Chemical, USA). Malondialdehyde (MDA) activity was determined by the method of Hartman [35]. Superoxide dismutase (SOD) activity was determined by the method of Zou et al. [36] (Assay kit; Cayman Chemical, USA). Glutathione peroxidase activity was assayed by the method of Paglia and Valentine [37]. (Assay kit; Cayman Chemical, USA).

2.11 Statistical Analysis
The data was presented as mean ± standard error of the mean. Results were analyzed statistically by One way ANOVA followed by Duncan’s multiple comparison test using the statistical package – SPSS version 20. Values were considered statistically significant at P<0.05

3. Results
Acute toxicity studies (LD50)
The acute toxicity test at 5000 mg/kg body weight of methanolic leaves extract of Cassia arereh produced no mortality after 48 hrs of observation. The median lethal dosage (LD50) of the methanolic leaves extract was therefore estimated to be greater than 5000mg/kg body weight. The extract did not produce any negative behaviour changes such as restlessness, excitement, respiratory distress, coma. Thus, the estimated LD50 indicated that the extract is safe.

| LD50 Determination of Methanolic Leaves Extract of Cassia arereh |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Dose            | Groups          | No. of Animal   | No. of Death   |
| 5000            | A               | 1               | 0              |
| 5000            | B               | 1               | 0              |
| 5000            | C               | 1               | 0              |
| 5000            | D               | 1               | 0              |
| 5000            | E               | 1               | 0              |

Phytochemical Screening
Phytochemical constituents of methanolic leaves extract of Cassia arereh revealed the presence of Flavonoids, Tannins, Saponins, Alkaloids, Cardiac glycosides, Steroids, Glycosides, Saponin glycosides, Anthraquinone glycosides, Terpenes, Anthraquinones, Cyanogenic glycosides and Volatile oils. Saponin glycosides are the most abundant phytochemical.

Table 2: Qualitative phytochemical screening of methanolic leaves extract of Cassia arereh

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Saponin Glycosides</td>
<td>+++</td>
</tr>
<tr>
<td>Anthraquinone Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>ND</td>
</tr>
</tbody>
</table>

Key
+= presence in trace quantity
++= presence in moderate
+++ = presence in large quantity
ND = Not detected

Table 3 showed the effects of treatment with methanolic leaves extract of Cassia arereh on serum liver function biochemical parameters in rats with CCl4 induced liver damage. The CCl4 treated rats have significantly elevated serum levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total bilirubin (TB), Direct bilirubin (DB), Alkaline phosphatase (ALP), Gamma Glutamyl Transferase (GGT) compared to normal control. Whereas, in CCl4 treated rats, Albumin (ALB) and Total protein (TP) levels were significantly reduced. Methanolic leaves extract of Cassia arereh at the doses of 30mg/kg, 100mg/kg, 150mg/kg and 200mg/kg, and also silymarin at 100mg/kg significantly reduced the elevated serum enzymes markers and increased the levels of Total protein and Albumin.

| Table 3: Effect of methanolic leaves extract of Cassia arereh on serum liver function biochemical parameters in rats with CCl4 induced hepatotoxicity |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| GROUP           | ALB (g/dl)      | TP (g/dl)       | ALT (U/L)       | AST (U/L)       | TB (mg/dl)      | DB (mg/dl)      | ALP (U/L)       | GGT (U/L)       |
| Group I         | 3.73±0.4        | 4.25±0.02       | 54.59±0.33      | 127.82±0.84     | 0.79±0.01       | 0.41±0.09       | 108.12±7.08     | 36.60±2.66      |
| Group II        | 2.23±0.31a      | 1.52±0.05a      | 79.22±0.43      | 206.94±3.18a    | 2.14±0.03a      | 1.23±0.06a      | 124.94±2.31a    | 107.00±5.5a     |
| Group III       | 4.55±0.99b      | 2.51±0.12b      | 72.91±0.31b     | 167.02±0.49b    | 1.66±0.02b      | 0.70±0.18b      | 110.96±8.12b    | 73.00±4.11b     |
| Group IV        | 3.34±0.16c      | 3.75±0.04c      | 67.61±0.52c     | 133.03±1.45c    | 1.20±0.02c      | 0.61±0.02c      | 101.46±8.71c    | 46.80±1.24c     |
| Group V         | 3.40±0.07d      | 2.07±0.23d      | 70.49±0.84d     | 131.20±1.11d    | 1.32±0.14d      | 0.68±0.05d      | 114.29±1.29d    | 74.00±2.35d     |
| Group VI        | 3.40±0.07e      | 2.07±0.23e      | 72.59±0.53e     | 183.62±1.47e    | 1.78±0.01e      | 0.80±0.08e      | 112.46±0.83e    | 72.80±2.78e     |
| Group VII       | 3.73±0.21f      | 3.88±0.03f      | 62.65±0.39f     | 126.26±1.70f    | 0.89±0.02f      | 0.64±0.03f      | 93.97±12.20f    | 43.20±2.20f     |

Values are expressed as mean ± Standard error of mean. Mean values having different superscript letters in a column are significantly different (p<0.05) (one-way ANOVA followed by Duncan’s multiple ranges multiple comparison). Key: ALB- Albumin, TP- Total protein, ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, TB- Total bilirubin, DB- Direct bilirubin, ALP- Alkaline phosphatase, GGT - Gamma Glutamyl Transferase Group I: received liquid paraffin (1ml/kg body weight peros) Group II: received 1ml/kg body weight i.p of 30% CCl4 in liquid paraffin for every 72 hrs for 14 days Group III: received 50 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30% CCl4 in liquid paraffin for every 72 hrs for 14 days Group IV: received 100 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30% CCl4 in liquid paraffin for every 72 hrs for 14 days Group V: received 150 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30% CCl4 in liquid paraffin for every 72 hrs for 14 days Group VI: received 200 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30% CCl4 in liquid paraffin for every 72 hrs for 14 days Group VII: received 100 mg/kg peros of silymarin once daily and 1ml/kg body weight of 30% CCl4 in liquid paraffin for every 72 hrs for 14 days.

~ 1348 ~
Table 4 represents the effect of methanolic leaves extract of *Cassia arereh* on liver homogenate enzymatic antioxidants activities. The enzymatic antioxidants [Catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD)] activities were significantly reduced in the induced non treated group. Treatments with methanolic leaves extract of *Cassia arereh* at the doses of 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg, and silymarin at 100mg/kg significantly elevated the enzymatic antioxidant activities.

**Table 4**: Effect of methanolic leaves extract of *Cassia arereh* on liver homogenate enzymatic antioxidants activities in rats with CCl4-induced hepatotoxicity.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>GPx (nmol/min/ml)</th>
<th>SOD (U/mg)</th>
<th>CAT (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>46.2±3.47</td>
<td>91.67±0.60</td>
<td>295.93±12.23</td>
</tr>
<tr>
<td>Group II</td>
<td>25.10±1.13</td>
<td>56.60±1.25</td>
<td>149.21±3.22</td>
</tr>
<tr>
<td>Group III</td>
<td>76.80±15.78</td>
<td>81.25±0.60</td>
<td>179.77±2.35</td>
</tr>
<tr>
<td>Group IV</td>
<td>51.48±7.05</td>
<td>97.57±0.35</td>
<td>200.72±2.68</td>
</tr>
<tr>
<td>Group V</td>
<td>40.24±4.39</td>
<td>94.79±0.60</td>
<td>178.56±4.06</td>
</tr>
<tr>
<td>Group VI</td>
<td>51.22±16.10</td>
<td>71.18±0.92</td>
<td>162.00±4.58</td>
</tr>
<tr>
<td>Group VII</td>
<td>71.98±14.33</td>
<td>92.71±1.60</td>
<td>259.76±21.35</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard error of mean. Mean values having different superscript letters in a column are significantly different (p<0.05) (one-way ANOVA followed by Duncan’s multiple ranges multiple comparison).

Lastly, table 6 showed the effects of treatment with methanolic leaves extract of *Cassia arereh* on serum antioxidant vitamins in rats with CCl4 induced liver damage. The CCl4 treated rats have significantly lower serum levels of vitamin C and E. Treatments with methanolic leaves extract of *Cassia arereh* at the doses of 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg, and silymarin at 100mg/kg significantly elevated levels of antioxidant vitamins (E and C).

**Table 6**: Effect of Administration of methanolic leaves extract of *Cassia arereh* on serum Vitamin C and E in rats with CCl4 induced hepatotoxicity.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH (mg/ml)</td>
<td>MDA (µmol/l)</td>
</tr>
<tr>
<td>Group I</td>
<td>4.5±0.12</td>
<td>6.29±0.09</td>
</tr>
<tr>
<td>Group II</td>
<td>0.91±0.11</td>
<td>11.07±0.20</td>
</tr>
<tr>
<td>Group III</td>
<td>2.38±0.09</td>
<td>8.61±0.13</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.77±0.34</td>
<td>7.75±0.08</td>
</tr>
<tr>
<td>Group V</td>
<td>3.12±0.33</td>
<td>10.03±0.11</td>
</tr>
<tr>
<td>Group VI</td>
<td>1.55±0.05</td>
<td>8.44±0.24</td>
</tr>
<tr>
<td>Group VII</td>
<td>4.02±0.11</td>
<td>7.47±0.16</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard error of mean. Mean values having different superscript letters in a column are significantly different (p<0.05) (one-way ANOVA followed by Duncan’s multiple range test).

**Table 5**: Effect of Administration of methanolic leaves extract of *Cassia arereh* on serum Vitamin C and E in rats with CCl4 induced hepatotoxicity.

**Table 5** represent the results of methanolic leaves extract of *cassia arereh* on serum and liver homogenate oxidative stress markers. Malondialdehyde (MDA) which shows the level of lipid peroxidation was increased in CCl4 treated rats when compared with normal group in both the serum and liver homogenate. This was accompanied by decrease in reduced glutathione (GSH) levels in both the serum and liver homogenate. Treatments with methanolic leaves extract of *Cassia arereh* at the doses of 50 mg/kg, 100 mg/kg, 150 mg/kg and 200 mg/kg, and silymarin at 100 mg/kg significantly elevated the GSH levels and decreased the elevated level of MDA in both serum and liver homogenate.
for 14 days Group IV: received 100 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30 % CCl₄ in liquid paraffin for every 72 hrs for 14 days Group V: received 150 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30% CCl₄ in liquid paraffin for every 72 hrs for 14 days Group VI: received 200 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30 % CCl₄ in liquid paraffin for every 72 hrs for 14 days Group VII: received 100 mg/kg peros of silymarin once daily and 1ml/kg body weight of 30 % CCl₄ in liquid paraffin for every 72 hrs for 14 days

4. Discussion

There was no lethal effect or toxicity observed after a single oral administration of methanolic leaves extract of Cassia arereh for the 14 days of the experiment. Negative behavioural changes such as restlessness, excitement, respiratory distress and coma were not observed. According to Loomis and Hayes classification [38], a test substance administered orally and having an LD₅₀ within the range of 5000–15000 mg/kg is considered as practically non-toxic. The estimated LD₅₀ of the leaves extract of Cassia arereh being found in this range suggests that the plant should be regarded as practically non-toxic in acute ingestion.

The hepatoprotective effect of several medicinal plants has been attributed to the presence of chemical constituents like flavonoids, alkaloids, phenols, essential oil, carotenoids and glycosides [39, 40, 41]. The preliminary phytochemical screening revealed the presence of some of these phytochemicals in methanolic leave extract of Cassia arereh. Specifically, flavonoids, alkaloids, tannins, saponins, saponin glycosides, glycosides, cardiac glycosides, steriods, anthraquinone glycosides, terpenes, anthraquinones and cyanogenic glycosides. Flavonoids are polyphenolic compounds that are ubiquitous in nature [42]. Research indicates that flavonoids and other phenolic compounds play a role in scavenging free radicals and in the inhibition of lipid peroxidation [43, 44]. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation [45]. Saponins are also believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability [45]. Plant phenolics are one of the major groups of compounds acting as primary antioxidant free radical terminators. Cardiac glycosides are important class of naturally occurring agents whose actions helps in the treatment of congestive heart failure [46]. Cardiac glycosides have been shown to aid in the treatment of congestive heart failure and cardiac arrhythmia [46]. Anthraquinone glycosides exhibits antifungal properties, inhibit excessive renal tubular cell proliferation, delay deterioration of patients in renal failure and modulate inflammation by partially inhibiting cyclooxygenase [47].

Liver is one of the largest organs in human body and the major site for intense metabolism and excretion [48]. It is involved with almost all the biochemical pathways responsible for growth, fight against disease, nutrient supply, energy provision and reproduction [49]. Liver damage inflicted by hepatotoxic agents is of grave consequences [50]. Hepatotoxic chemicals cause damage to the liver cells mainly by inducing lipid peroxidation and other oxidative events [51]. Liver cell injury caused by various toxicants such as carbon tetrachloride and paracetamol is well-studied. In the present work, CCl₄ treated rats showed significant elevation in AST, ALT, ALP, GGT, total and direct bilirubin as compared with that of normal as well as the extract and silymarin treated groups. There was remarkable decrease in total protein and albumin level. Being a potent hepatotoxin, CCl₄ is the most extensively used chemical agent for investigation of hepatoprotective activity on various experimental animal model [52]. The hepatotoxic effects caused by CCl₄ are due to the formation of free radicals during its metabolism by hepatic microsme, which in turn, cause lipid peroxidation of the cellular membrane leading to the necrosis of hepatocyte. AST, ALT and ALP are the serum hepatobiliary enzymes present normally in the liver in high concentrations. Upon hepatic dysfunction or damage these enzymes will be leaked into the circulation; raising serum concentration of these enzymes. Elevated levels of serum AST and ALT are due to alteration or increase in the permeability of the hepatocyte membrane and increased synthesis or decreased catabolism of aminotransferases [53]. Increase in serum alkaline phosphatase is due to increased synthesis in the presence of increasing biliary pressure [54]. Therefore, the elevated serum level of AST, ALT and ALP in CCl₄ treated animals indicated cellular breakage and loss of functional integrity of cell membranes and increased biliary pressure in the liver. GGT, a membrane bound enzyme is a well-known indicator of cell and tissue damage by toxic substances. The substantial increase in the CCl₄-intoxicated rats is a further indication of liver damage. Bilirubin is a useful index of the excretory function of the liver. Elevated level of serum conjugated bilirubin implies regurgitation of bilirubin glucuronides from hepatocytes back into plasma, usually because of intrahepatic or extrahepatic obstruction to bile outflow and cholestasis [55]. It may also be an indication of erythrocytes degradation caused due to liver injury. It is also a further indication of liver cell impairment [55]. One of the most important functions of the liver is protein synthesis. Albumin is a major part of the total protein made specifically by the liver. Liver damage causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein [56]. Decreased total protein level including albumin levels are due to defective protein biosynthesis arising from hepatocellular injury. The CCl₄ intoxication caused disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein [56]. In this study, CCl₄ significantly decreased serum total protein and albumin content which is a further indication of hepatocellular damage [56].

The methanolic leaves extract of Cassia arereh at 50, 100, 150 and 200 mg/kg showed hepatoprotective activity by reducing CCl₄-induced elevated levels of AST, ALT, ALP, GGT, total and direct bilirubin and increased CCl₄-induced reduction of serum albumin and total protein. Reduction in the levels of AST and ALT is an indication of regeneration process of hepatocytes [57]. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stabilization of the biliary function [57]. The ability of methanolic extract of Cassia arereh to significantly reduce the level of serum total and direct bilirubin may also suggest the potential of the extract in clearing bilirubin from the serum when its level elevated. The increase in protein and albumin levels is an indication of stabilization of endoplasmic reticulum leading to protein synthesis [58]. The hepatoprotective effect of the extract overall at 100mg/kg was comparable with that of silymarin a known hepatoprotective agent. Silymarin, a mixture of polyphenolic flavonoids, derived from the fruits and seeds of Silybum marianum (milk thistle) is one of the most commonly used hepatoprotective and antioxidant drug [59]. Silymarin has both hepatoprotective and regenerative actions. The mechanisms of these effects are
reduction of free radicals formed by toxins that damage the cell membrane and competitive inhibition through hepatocyte external cell membrane modification [60]. Silymarin forms a complex that impedes the entrance of toxins into the interior of liver cells. Additionally, silymarin metabolically stimulates hepatic cells and activates the RNA synthesis of ribosomes to stimulate protein formation [61]. These results suggest that the leaves extract of Cassia arereh possibly protect the structural integrity of the cell membrane of hepatocytes or enhance regeneration of damaged liver cells via a mechanism similar to that of silymarin.

Lipid peroxidation has been implicated in the destructive process of liver injury caused by CCl₄ administration [62]. The present study showed a significant increase in the level of MDA in both serum and liver homogenate of CCl₄-treated rats when compared with the control groups. This increase in MDA levels suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [63]. This in turn alters the ratio of polyunsaturated to other fatty acids, thus, leading to a decrease in the membrane fluidity which may be sufficient to cause cell death [64]. The impairment of the antioxidant defense system is considered as a critical event in CCl₄-induced hepatotoxicity. Marked depletion in non-enzymatic antioxidants including GSH, Vitamin C and E of CCl₄ treated rats accompanied by significant inhibition of enzymatic antioxidants including GPx, SOD and catalase activities was observed in the present study. GSH is a sulfhydryl peptide enormously present in all biological systems. It forms the first line of defense against oxidative insult by acting as a non-enzymatic antioxidant by direct interaction of its sulfhydryl group with ROS or it can be involved in the enzymatic detoxification reaction of ROS as a cofactor or as a coenzyme [64]. Vitamin C and E play essential roles in scavenging oxygen-derived free radicals. SOD is a metalloprotein that catalyses the dismutation of superoxide radicals [65]. Catalase is a heme protein which catalyses the reduction of H₂O₂ to water and oxygen and thus protects the cell from oxidative damage by H₂O₂ and OH [66]. Glutathione peroxidase is a seleniumzyme, which plays a major role in the reduction of H₂O₂ and hydroperoxide to non-toxic products. It has been well reported that acute administration of CCl₄ induces oxidative stress in rats [67]. The pathogenesis of oxidative stress induced hepatotoxicity could be due to either increased generation of reactive oxygen species and/or depletion of the antioxidants enzymes in the defence system. Therefore, the observed decline in the level of GSH, Vitamin C and E, GPx, CAT and SOD in CCl₄-treated rats suggests enhanced lipid peroxidation during tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals [68, 69].

In the present study, the administration of methanolic leave extract of Cassia arereh significantly reduced CCl₄ induced MDA elevation and increased GSH, Vitamin C and E level, GPx, SOD and CAT activities. The results are comparable with those of silymarin treated groups. Phytochemical screening of Cassia arereh revealed the presence of flavonoids, glycosides, alkaloids, antraquinone, steroids and tannins. Phytochemicals like the flavonoids, triterpenoids, saponins, and alkaloids are known to possess hepatoprotective activity [70, 71]. Flavonoids have been reported to possess strong antioxidant activity and provokes free radical scavenging enzyme system, thus may protect against oxidative stress caused by hepatotoxic agents [72]. The reduction in MDA level, elevation of GSH, vitamin C and E, enhanced activities of SOD, CAT and GPx in the liver of CCl₄-treated rats might be to the inhibitory effect of the extract on lipid peroxidation and ability to antagonize the formation of free radical damage caused by CCl₄. Hence, it is likely that the mechanism hepatoprotection of leave extract of Cassia arereh is due to its antioxidant effect.

5. Conclusion

The methanolic leaves extract of Cassia arereh showed a significant protection against CCl₄-induced liver damage as observed from the results of the present study. The probable mechanism of hepatoprotection is antioxidant activity. The antioxidant activity of the extract may be attributed to the presence of phytochemicals detected in the plant.

Further work on this plant is needed to established safeness of the plant for long term usage (Chronic and subchronic toxicity evaluation). Isolation and characterization of the active ingredients responsible for the hepatoprotective effect of the methanolic leaves extract of Cassia arereh can also be explore.

6. References

55. Wolf PL. Clinical significance of an increased or decreased serum alkaline phosphatase; Arch pathol lab med. 1978; 102:497-501.