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Hepatoprotective and antioxidant activity of methanolic leaves extract of *Cassia arereh* in CCl_4-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 CCl_4 - 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 CCl_4 – 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 variedly 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, Superoxde 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, Balanites aegyptiaca, Khaya senegalensis* [13] (Baytop, 1999), and most times are found to offer significant relief [14].

Cassia arereh is a small tree which belongs to the family Caesalpiniaceae. 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 Babur-Bura and Maraguwa in Kare-kare languages [15]. It is found also in northern Nigeria, Cameroun, Ethopia, and Eritrea. Almost all parts of the plant are used locally as medicine [16]. It is used in Nigerian traditional medicine for the treatment of diarrhoea, dysentery, dermatitis, malaria, skin infections and liver disease [16].

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 45^0C and the percentage yield was calculated. The extracts were stored in a closed container until required for reconstitution in distilled water (for oral administration).

2.6 Phytochemical Analysis
Volatile oils, tannins, were estimated by the method of Harborne [18], terpenes and steriods 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 Kocipai [22].

2.7 acute toxicity studies (Determination of ld_{50})
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 LD_{50} is less than 5000mg/kg and if one (1), two (2) or none animal died within 48hrs, the LD_{50} 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 CCl_4 induction for every 72 hrs.
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 CCl_4 induction for every 72 hrs.
The animals were sacrificed after 48 hrs of CCl_4 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 *et al*. [26] Total and conjugated bilirubin was determined using the method of Jendrassik and Grof [27] (Assay kit: Randox laboratories, UK), Alkaline phosphatase was estimated using Colorimetric method of Sood [28] (Assay kit: Randox laboratories, UK), Albumin was determined by the dye binding technique utilizing Bromocresol green (BCG) as modified by Doumas *et al*. [29] 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 (ld$_{50}$)

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 (LD_{50}) of the methanolic leaves extract was therefore estimated to be greater than 5000mg/kg body weight. The extract did not produce any negative behavioural changes such as restlessness, excitement, respiratory distress, coma. Thus, the estimated LD_{50} indicated that the extract is safe.

Table 1: LD_{50} Determination of Methanolic Leaves Extract of Casssia 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, Steriods, 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*

Phytochemical constituents	Status
Flavonoids	++
Tannins	++
Saponins	++
Alkaloids	++
Cardiac Glycosides	++
Steroids	++
Glycosides	++
Saponin Glycosides	+++
Anthraquinone Glycosides	+
Terpenes	+
Resins	+
Anthraquinones	+
Cyanogenic glycosides	+
Volatile oils	ND

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 CCl_4- induced liver damage. The CCl_4 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 CCl_4 treated rats, Albumin (ALB) and Total protein (TP) levels were significantly reduced. Methanolic leaves extract of *Cassia arereh* at the doses of 50mg/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 CCl_4 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±0.31^{b}	1.52±0.05^{d}	79.22±0.43^{d}	206.94±3.18^{d}	2.14±0.03^{d}	1.23±0.06^{c}	124.94±2.31^{c}	107.00±5.5^{c}
Group III	3.55±0.09^{a}	2.51±0.12^{b}	72.91±0.31^{d}	167.02±0.49^{b}	1.66±0.02^{c}	0.70±0.18^{b}	110.96±8.12^{b}	73.00±4.11^{b}
Group IV	3.34±0.16^{a}	3.75±0.04^{a}	67.61±0.52^{b}	133.03±1.45^{a}	1.20±0.02^{b}	0.61±0.02^{a}	101.46±8.71^{a}	46.80±1.24^{a}
Group V	3.40±0.07^{a}	2.07±0.23^{c}	70.49±0.84^{c}	131.20±1.11^{a}	1.32±0.14^{b}	0.68±0.05^{a}	114.29±1.29^{b}	74.00±2.35^{b}
Group VI	3.40±0.07^{a}	3.82±0.05^{a}	72.59±0.53^{d}	183.69±14.7^{c}	1.78±0.01^{c}	0.80±0.08^{b}	112.46±0.83^{b}	72.80±2.78^{b}
GroupVII	3.73±0.21^{a}	3.88±0.03^{a}	62.65±0.39^{a}	126.26±1.70^{a}	0.89±0.02^{a}	0.64±0.03^{a}	93.97±12.20^{a}	43.20±2.20^{a}

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% CCl_4 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 % CCl_4 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 % CCl_4 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% CCl_4 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 % CCl_4 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 % CCl_4 in liquid paraffinfor every 72 hrs for 14 days.

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)] a ctivitieswere 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 antioxidants activities.

Table 4: Effect of methanolic leaves extract of *Cassia arereh* on liver homogenate enzymatic antioxidants activities in rats with CCl_4-induced hepatotoxicity

GROUP	GPX(nmol/min/ml)	SOD (U/mg)	CAT(U/ml)
Group I	46.26±3.47	91.67±0.60	295.93±12.23
Group II	25.10±1.13^{c}	56.60±1.25^{e}	149.21±3.22^{d}
Group III	76.80±15.78^{a}	81.25±0.60^{c}	179.77±6.91^{b}
Group IV	51.48±7.05^{a}	97.57±0.35^{a}	200.72±2.68^{b}
Group V	40.24±4.39^{b}	94.79±0.60^{a}	178.56±4.06^{b}
Group VI	51.22±16.10^{a}	71.18±0.92^{d}	162.00±4.58^{c}
Group VII	71.98±14.33^{a}	92.71±1.60^{b}	259.76±21.35^{a}

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: SOD-** superoxide dismutase, **GPx-** Glutathione peroxidase, **MDA-** Malondialdehyde, **GSH-** Reduced glutathione, **CAT-** Catalase **Group I:** received liquid paraffin (1ml/kg body weight peros) **Group II:** received 1ml/kg body weight i.p of 30% CCl_4 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 % CCl_4 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 % CCl_4 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% CCl_4 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 % CCl_4 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 % CCl_4 in liquid paraffinfor every 72 hrs for 14 days

Table 5 represent the result 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 CCl_4 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.

Table 5: Effect of Administration of methanolic leaves extract of *Cassia arereh* on serum and homogenate oxidative stress status in rats with CCl_4 induced hepatotoxicity

GROUP	Serum		Liver	
	GSH(mg/ml)	MDA(μmol/l)	GSH (mg/ml)	MDA (μmol/l)
Group I	4.54±0.12	6.29±0.09	10.75±0.06	4.16±0.59
Group II	0.91±0.11^{e}	11.07±0.20^{d}	6.03±0.15^{c}	9.06±0.57^{c}
Group III	2.38±0.09^{c}	8.61±0.13^{b}	7.63±0.54^{a}	4.75±1.19^{b}
Group IV	2.77±0.34^{b}	7.75±0.08^{a}	9.92±0.36^{a}	3.81±0.42^{a}
Group V	3.12±0.33^{b}	10.03±0.11^{c}	6.81±0.36^{b}	4.53±0.41^{a}
Group VI	1.55±0.05^{d}	8.44±0.24^{b}	9.15±2.91^{a}	6.28±0.46^{b}
Group VII	4.02±0.11^{a}	7.47±0.16 ^{a}	10.65±0.38^{a}	3.51±0.51^{a}

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). **Key: GSH-** Reduced glutathione, **MDA-**Malondialdehyde Catalase **Group I:** received liquid paraffin (1ml/kg body weight peros) **Group II:** received 1ml/kg body weight i.p of 30% CCl_4 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 % CCl_4 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 % CCl_4 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% CCl_4 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 % CCl_4 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 % CCl_4 in liquid paraffinfor every 72 hrs for 14 days

Lastly, table 6 showed the effects of treatment with methanolic leaves extract of *Cassia arereh* on serum antioxidant vitamins in rats with CCl_4 induced liver damage. The CCl_4 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 CC_4 induced hepatotoxicity

GROUP	Vit C (μmol/l)	Vit E(μmol/l)
Group I	336.41±1.35	92.65±0.27
Group II	183.52±2.88^{e}	29.12±2.96^{f}
Group III	301.89±1.76^{c}	52.50±0.23^{e}
Group IV	287.47±3.00^{c}	88.22±0.61^{b}
Group V	274.98±1.04^{d}	73.61±0.23^{c}
Group VI	332.85±5.84^{b}	65.08±0.20^{d}
Group VII	408.83±20.02^{a}	96.09±0.33^{a}

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). **Key: VitC-** Vitamin C **Vit E-**Vitamin E **Group I:** received liquid paraffin (1ml/kg body weight peros) **Group II:** received 1ml/kg body weight i.p of 30% CCl_4 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 % CCl_4 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 % CCl_4 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% CCl_4 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 % CCl_4 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 % CCl_4 in liquid paraffinfor 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 LD_{50} within the range of 5000–15000 mg/kg is considered as practically non-toxic. The estimated LD_{50} 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, CCl_4 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, CCL_4 is the most extensively used chemical agent for investigation of hepatoprotective activity on various experimental animal model [52]. The hepatotoxic effects caused by CCl_4 are due to the formation of free radicals during its metabolism by hepatic microsome, which in turn, cause lipid peroxidation of the cellular membrane leading to the necrosis of hepatocyte. AST, ALT and ALP are the serum hepatobillary 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 CCL_4 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 CCl_4-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 CCl_4 intoxication caused disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein [56]. In this study, CCl_4 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 CCl_4-induced elevated levels of AST, ALT, ALP, GGT, total and direct bilirubin and increased CCl_4-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 membranes 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 CCl_4 administration [62]. The present study showed a significant increase in the level of MDA in both serum and liver homogenate of CCl_4-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 [62]. 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 [63].The impairment of the antioxidant defense system is considered as a critical event in CCl_4-induced hepatotoxicity. Marked depletion in non-enzymatic antioxidants including GSH, Vitamin C and E of CCl_4 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 hemeprotein which catalyses the reduction of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and OH [66]. Glutathione peroxidase is a selenoenzyme, which plays a major role in the reduction of H_2O_2 and hydroperoxide to non-toxic products It has been well reported that acute administration of CCl_4 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 CCL_4-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 CCL_4 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, anthraquinone, 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 [71]. The reduction in MDA level, elevation of GSH, vitamin C and E, enhanced activities of SOD, CAT and GPx in the liver of CCL_4-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 CCl_4. 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 CCl_4- 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

1. Ward FM, Daly MJ. Hepatic disease. In: Clinical Pharmacy and Therapeutics (Walker R. and C. Edwards Eds.). Churchill Livingstone, New York, 1999, 195-212.
2. Subramonium A, Pushpangadan P. Development of phytomedicines for liver diseases. Indian J. Pharmacol. 1999; 31:166-175.
3. Sheweita SA, Abd El- Gabar M, Bastawy M. Carbon tetrachloride – induced changes in the activity of phase II drug metabolizing enzyme in the liver of male rats: role of antioxidants. Toxicology. 2001; 165(2):217.
4. Kataki MS, Ahmed MZ, Awasthi D, Tomar B, Mehra P, Yadav RS, *et al*. *In vitro* antioxidant profile of *Wedelia calandulaceae* leaves, Pharmacologia. 2012 3(3):75-83.
5. Odugbemi T. Medicinal Plants as Antimicrobials In: Outline and pictures of medicinal plants from Nigeria. University of Lagos press, 2006, 53-54.
6. Karan M, Vasisht K, Handa SS. Antihepatoxic activity of *Swerita chirata* on carbon tetrachloride induced hepatotoxicity in rats. Phyto. Res. 2009; 13:24-30.
7. Chatterjee TK. Medical plants with hepatoprotective properties. In herbal options, 3rd (ed), Calcutta Books and Allied publisher Ltd, 2000, 135-137.
8. Gamaniel KJ. Toxicity from medicinal plantd and their products. Nig. J. Vat. Pro. Medi. 2000; 4:4-7.
9. Tor-Anyiin TA, Sha'ato R, Oluma HOA. Phytochemical sceening and antibacterial activity of *Cissampeo mucronata*. A rich extract J. Pharm. Biores. 2006; 3(2):103-106.
10. Abdulrahman FL, Akan JC, Sodipo OA, Onyeyili PA. Effect of aqueous root-bark extract of *vitex domina sweet* on haematological parameters in rats. J. AM. Sci. 2010; 6(12):8-12.
11. Nostrol A, Germano MP, D'angelo V, Marino A, Cannatelli MA. Extraction on methods and biaautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 2000; 30:379-384.
12. Hikino H, Kiso Y. Natural products for liver diseases, Econ Med Plant Res. 1998; 2:39-72.
13. Baytop T. Therapy with medicinal plants in Turkey. 2nd edition, Nobel Medical Publish, Istanbul. Ltd, 1999, 533-534.
14. Arulkumaran KS, Rajasekaran R, Ramasamy M, Jegadeesan S, Kavimani A, Somasundaram D. *Cassia*

Roxburghii seeds protects liver against toxic effects of ethanol and carbon tetrachloride in rats. Int. J. Pharm Tech Res. 2009; 1(2):237-246.

15. Gambo MK, Karofi MUN. Karekare-English-Hausa dictionary. Ed.Schuh R G ©Yobe Language Research Project. Ajami presspublishers, Potiskum, Nigeria, 2004, 38.
16. Arbonnier M. Trees, shrubs and lianas of West African Dry Zones (IRAD, Margraf publishers GMBH MNHN, Cote D' Ivorie, 2004, 194.
17. Laure BKK, Thibau FT, Emmanuel TB, Jacques Kouam. Evaluation of antioxidant activity and protective effect of Cassia arereh (caesalpiniaceae) extracts against free radicalinduced oxidative haemolysis Int J Pharm. 2015; 5(4):1026-1036.
18. Harborne JB. Phytochemical methods. A guide to Modern Techniques of Plant Analysis 2nd edition. Chapman and Hall London, 1973, 1-10.
19. Sofowora A. African Medicinal plants. University of Ife Press (Nig). 3rd edition, 1973, 21-30.
20. Trease GE, Evans WC. A textbook of pharmacognosy. 11th Edition, Bailliere Tindall, London, 1978, 530.
21. El-Olemyl MM, Fraid JA, Abdulfattah AA. Experimental photochemistry, a laboratory manual. College of pharmacy, King Saud University, Saudi Arabia, 1994, 1-134.
22. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannin from leaves of Hawaiian *Vccinium vaticulatum and V. calycinium*. Pacific Sci. 1994; 48:458-463.
23. OECD. Guideline for testing chemicals. Acute toxicity-up and down procedure. 2001; 425:1-26.
24. Guntupalli MM, Chandana VR, Palpu P, Anine S. Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. J. Ethnopharmacol. 2006; 103(3):484-490.
25. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. chlin. Pathol. 1957; 28:56-63.
26. Gomall AG, Bardawill CS, David MM. Determination of Serum proteins by means of the biuret reaction. J. Biol. Chem. 1949; 177(2):751-766.
27. Jendrassik L, Grof P. Simplified photometric methods for the determination of bilirubin. Biochem. Zchr. 1938; 297:81-89.
28. Sood R. Medical laboratory technology. Method and interpretation.sed. Jaypee brothers medical publishers ltd, 1999, 488-490.
29. Doumas BT, Watson W, Briggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acda. 1971; 3:187-96.
30. Szasz G, Bergmeyer HU. A Kinetic Photometric Method for serum gamma glutamyl transpeptidase. J Clin. Chem. 1974: 15(1):124-126.
31. Rutkowski M, Grzegorczyk K. Kolorymetryczne oznaczanie stężenia witaminy C wosoczu krwi przy użyciu odczynnika fosforowolframianowego – mody fikacja metody Kyawa [Colorimetric determination of vitamin C concentration in blood plasma with phosphotungstate reagent – a modification of Kyaw method]. Diagn. Lab. 1998; 34:243 [in Polish].
32. Rutkowski M, Grzegorczyk K, Paradowski MT. Kolorymetryczna metoda oznaczania całkowitej witaminy E w osoczu krwi – modyfikacja własna metody Tsena [Colorimetric method of blood plasma total vitamin E determination – the own modification of Tsen method]. Diagn. Lab. 2005; 41:375 [in Polish].
33. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 1952; 195:130-140.
34. Patterson JW, Lazarow A. Methods of biochemical analysis. Glick A. ed New York, Inter Science. 1955; 2:259.
35. Hartman PE. Putative Mutagens and Carcinogens in Foods, Iv. Malondialdehyde. Environ. Mutaen. 1983; 5(4):603-607.
36. Zou GL, Gui XF, Zhong XL, Zhu YF. Improvements in pyroggallol autoxidation method for the determination of SOD activity. Progress Biochem Biophys, 1986, 71-73.
37. Paglia DE, Valentine WN. Studies on the quantitative and qualitative of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 1967; 70:158-169.
38. Loomis TA, Hayes AW. Loomis's Essentials of Toxicology, 4th ed. Academic Press, CA, 1996, 67-87.
39. Arulkumaran KS, Rajasekaran R, Ramasamy M, Jegadeesan S, Kavimani A, Somasundaram D. *Cassia Roxburghii* seeds protects liver against toxic effects of ethanol and carbon tetrachloride in rats. Int. J. Pharm Tech Res. 2009; 1(2):237-246.
40. Achuthan CR, Babu BH, Padikkala J. Antioxidant and Hepatoprotective effects of Rosa damascene, J. Pharm. Bio. 2003; 41(1):357-361.
41. Agarwal SS. Development of hepatoprotective formulations from plant sources, Pharmacology and Therapeutics in the New Millennium, New Delhi, 2001, 357-358-3.
42. Pridham JB. In: Phenolics in Plants in Health and Disease, Pergamon Press, New York. 1960, 34-35.
43. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies A. J. Clin. Nutr. 2005; 81(1):317-325.
44. Allen RRL, Carson C, Kwik-Uribe EM, Evans JW. Daily consumption of a dark chocolate containing flavanols and added sterol esters affects cardiovascular risk factors in a normotensive population with elevated cholesterol. J. Nut. 2008; 138(4):725-731.
45. Rao KS, Mishra SH. Screening of anti-inflammatory and hepatoprotective activities of alantolactone isolated from the roots of Inularacemosa. Indian Drugs 1995; 34(10):571-5.
46. Okwu DE. Evaluation of the chemical composition of indigenous spices and flavouring agents. Global J. Pure Applied Sci. 2001; 7:455-459.
47. Brito-Arias M. Synthesis and characterization of glycosides. Springer. 2007, 26251-26252.
48. Ram VJ. Herbal preparations as a source of hepatoprotective agents. J. Drug News and Pers. 2001; 149(6):353-363.
49. Ward FM, Daly MJ. Hepatic disease. In: Clinical Pharmacy and Therapeutics (Walker R. and C. Edwards Eds.). Churchill Livingstone, New York, 1999, 195-212.
50. Guylon AC, Hall JE. In: Textbook of medical physiology, 11th (Saunders, Philadelphia), 2006, 859-864.
51. Dianzani MU, Muzia G, Biocca ME, Canuto RA. Lipid peroxidation in fatty liver induced by caffeine in rats. Inter. J. Tis. Reac. 1991; 13(1):79-85.
52. Lee BJ, Senevirathne M, Kim JS, Kim YM, Lee MS, Jeong MH, *et al*. Protective effect of fermented sea tangle against ethanol and carbon tetrachloride-induced hepatic

damage in Sprague-Dawley rats. Food & Chem Toxicol. 2010; 48:1123-1128.
53. Nuduka N. Clinical biochemistry for students of pathology, Longman Nigerian Plc, 1999, 1-236.
54. Moss DW, Butterworth PJ. Enzymology and Medicine. Pitman Medical, London, 1974, 139.
55. Wolf PL. Clinical significance of an increased or decreased serum alkaline phosphatase; Arch pathol lab med. 1978; 102:497-501.
56. Navarro VJ, Senior JR. Drug-related hepatotoxicity. New Eng. J. Medi. 2006; 354:731-739.
57. Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Protective effects of HD-03, A herbal formulation, against various hepatotoxic agents in rats. J. Ethnopharmac. 1998; 63(1):181-86.
58. Gupta MM, Azumder UK, Kumar RS, Sivakumar T, Gomathi P, Rajeshwar Y. Antioxidant defense system induced by a methanol extract of Caesapiniabonducella in rat liver. J. Pharmac. Bio. 2005; 43(5):411-419.
59. Abou Zid S. Silymarin, Natural Flavonoligans from Milk Thistle. In: Venketeshwer, R., editor. Phytochemicals- A Global Perspective of Their Role in Nutrition and Health. Rijeka: Croatia Intech, 2012, 255-272.
60. Trouillas P, *et al*. Mechanism of the antioxidant action of silybin and 2, 3- dehydrosilybin flavonoligans: a joint experimental and theoretical studt. J. Phys. Chem. 2008; 112(1):1054-1063.
61. Pietrangelo A, Gualdi R, Casalgrandi G, Montosi G, Ventura E. Molecular and cellular aspects of iron-induced hepatic cirrhosis in rodents. J. Clin. Invest. 1995; 95(18):24-31.
62. Jeon GI, Yoon MY, Park HR, Lee SC, Park E. Neuroprotective activity of Viola mandshurica extracts on hydrogen peroxideinduced DNA damage and cell death in PC12 cells. Ann New York Academy Sci; 2009; 1171:576-582.
63. Rotruck JT, Pope AL, Ganther H, Awanson AB, Haffeman DG, Hofekstra WG. Selenium: Biochemical role as a component of glutathione peroxides. J. of Sci. 1979; 179:588-590.
64. Sunitha S, Nagaraj M, Varalakshmi P. Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defense system in cadmium induced hepatotoxicity in rats. Fitoterapia. 2001; 72:516-23.
65. McCord JM, Fridovich I, Wang H. Superoxide dismutase, an enzymic function for erythrocuprein (Hemocuoprein). J. Biol Chem. 1976; 244(1):6049-6055.
66. Chance B, Greenstein DS, Roughton RJW. The mechanism of catalase action 1-steady state analysis. J. Arc. Bioch. Biophy. 1952; 37:301-39.
67. Alkreathy HM, Khan RA, Khan MR, Sahreen S. Hepatoprotective effect of *Spirulinalonar* on paracetamol induced liver damage in rats J. BMC Comp. Alt Med. 2014; 14:452.
68. Kuriakose GC, Kurup MG. Hepatoprotective effect of *Spirulinalonar* on paracetamol induced liver damage in rats. Asi. J. Exp. Bio. Sci. 2010; 1(3):614-623.
69. Showkat AG, Ehtishamul H, Akbar M, Mohmmad AZ. Amelioration of carbon tetrachloride induced oxidative stress in kidney and lung tissues by ethanolic rhizome extract of *Podophyllumhexandrum* in Wistar rats. J. Medi. Pla. Res. 2010; 4(16):1673-1677.
70. Tran QI, Adnyana IK, Tezuka Y, Nagaoka T, Tran QK, Kadota S. Triterpene saponins from Vietnamese ginseng (*Panax vietnamensis*) and their hepatocyte protective activity. J. of Nat. Prod. 2001; 64(1):456-61.
71. Tattini M, Galardi C, Pinelli P, Massai R, Remorini D, Agati G. Differential accumulation of flavonoids and hydroxyl cinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. J. of New Phytologist. 2004; 163:547-561.