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Economic Importance of Garcinia Kola: Evidences of Shielding Outcome Against Carbon Tetrachloride-Induced Kidney Toxicity in Experimental Models

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Abstract

Introduction: The aim of the study was to evaluate the hepatoprotective effect of aqueous extract of Garcinia kola) seeds on carbon tetrachloride (CCl4) induced liver damage in adult Wistar rats. Thirty adult Wistar rats weighing between 65-145g were randomly divided into six groups of 5 rats each. Group A (control) rats were orally administered with 5ml/kg body weight of distilled water daily for 2 weeks, group B rats were administered distilled water orally daily for 2 weeks (volume per body weight) and carbon tetrachloride (CCl4) 0.4 m1/kg intraperitonially as a single application on day 14 of the experiment. Group C rats were administered 100 mg of silymarin / kg body weight once daily for 2 weeks followed by a single dose of CCl4 (0.4 m1) on day 14 of the experiment. Group D rats were administered 400 mg aqueous extract bitter kola (Garcinia kola) / kg body weight orally once daily for 2 weeks. Group E and F rats were administered 400 mg and 200 mg aqueous extract bitter kola (Garcinia kola) seed) orally once daily for 2 weeks followed by a single dose of CCl4 (0.4 m1) on day 14 respectively. At the end of the experiment. Garcinia kola seed aqueous extract caused significant decrease in the aspartate aminotransferase (AST) levels in the serum of the extract treated groups. Histopathological examinations revealed distortion of histoarchitecture such as, sinusoidal congestion, necrosis, steatosis and fibrosis was observed in group B. The administration of aqueous extract of Garcinia kola seed remarkably inhibited histoarchitectural distortion induced by CCl4 administration. Effects of the extract at dose of 200mg/kg was comparable to the reference drug. Garcinia kola aqueous seed extract showed a remarkable hepatoprotective and antioxidant activity against CCl4 induced hepatotoxicity as observed from the serum marker enzymes and antioxidant levels in liver tissues. CCl4 induced a significant rise in AST, ALT, and ALP with an increase of superoxide dismutase (SOD), catalase (CAT) and reduction lipid peroxidation (MDA). Treatment of the rats with the extract significantly ($p<0.05$) altered serum marker enzymes and antioxidant levels to near normal compared with CCl4- treated rats (group B). The activity of the extract at dose of 400mg/kg (group F) was comparable to the standard drug confirmed by histopathological examinations of liver sections. ***Conclusion:*** *Garcinia kola* aqueous extract has hepatoprotective and antioxidant properties against CCl4-induced hepatotoxicity in Wistar rats.

Keywords: liver, carbon tetrachloride, hepatotoxicity, *Garcinia kola*

1. Introduction

According to Ahsan et al. (2009), liver disorders are among the deadliest illnesses in the world today and provide a significant threat to global public health. Viral hepatitis, alcoholic and non-alcoholic liver diseases, auto-immune liver diseases, metabolic liver disease, drug-induced liver damage, gallstones, etc. are a few of the more well-known liver problems. According to Franchesca et al. (2010), an increase in the prevalence of

drunkenness, drug usage (especially with hazardous substances), and other poor lifestyle choices including consuming fatty meals has contributed to the morbidity and mortality associated with liver illnesses.

According to Dianzani et al. (1991), the primary way that most hepatotoxic substances harm liver cells is by causing lipid peroxidation and other oxidative damages. Garcinia kola, often known as "Bitter Kola," is a member of the Guittiferae family. The plant has demonstrated pharmacological, antiviral, anti-microbial, and anti-inflammatory effects. In a system using animals as models, carbon tetrachloride (CC14) has been widely utilized to examine liver damage brought on by free radicals. According to studies on liver damage using CC14-treated rats, CC14 not only caused necrosis but also apoptosis in the organs (Shi et al., 1998; Sun et al., 2001).

Although the exact method by which CC14 damages the liver is unknown, multiple lines of evidence point to the possibility that free radical metabolites are to blame (Williams et al., 1990). Cytochrome P-450 performs a 1-electron reduction on CC14 to produce the trichloromethyl radical. Unsaturated fatty acids and the trichloromethyl radical react to form a fatty acid radical, which causes lipid peroxidation (Shi et al., 1998; Sun et al., 2001).

2. Materials and Methodology

2.1 Acquisition of Plant Materials

Garcinia kola seeds were obtained from modern market in Nigeria and was authenticated by a plant scientist in Nigeria.

2.2 Acquisition of Carbon Tetrachloride

Silymarin and Carbon tetrachloride ($CC1_4$) was obtained and authenticated by a university chemist in Nigeria.

2.3 Extraction of Plant Materials

Preparation of the plant (*Garcinia kola seeds*) extracts were peeled, sliced and dried in the air for 5 days. The dried, sliced feeds were ground into flour with a mortar and pestle. The extracts were then dissolved in 1.5 liters of distilled water and left to stand for a period of 48 hours after which it was sieved using a Whattman filter paper and the filtrate are refrigerated while the shaft is dried and weighed.

2.4 Preparation of Silymarin

52 tablets of 70mg of Silymarin was pounded using a laboratory mortar and pestle and was diluted in 1.5 liters of distilled water and the concentration of silymarin gotten was 2.4mg/ml.

2.5 Experimental Animal

Thirty (30) Wistar rats were housed in the Animal House of the Department of Human Anatomy and allowed to acclimatize for two weeks prior to the commencement of the experiments. All the animals were given food (rat chow) and water ad libitum. Experimental groups were given aqueous extract in an amount of 200mg/kg and 400mg/kg, in addition to a single dose of CCL4. The experimental rats were weighed at the beginning and at the end of the study.

3. Methodology

3.1 Experimental Design

A total number of 30 Wistar rats (male and female) were distributed randomly into **six** groups (six rats/group). The experiment lasted for a period of fourteen days during which.

Group A (negative control group) received normal saline, 5mls per body weight daily for 2 weeks.

Group B ($CC1_4$positive control group) received 0.4ml/kg on 'day 14'.

Group C (standard control group) received 100mg/kg of silymarin for 2 weeks followed by a single dose of $CC1_4$on day 14.

Group D (extract control group) received 400mg/kg for 2 weeks.

Group E (prophylactic treatment group) received 400mg/kg daily for 2 weeks followed by a single dose of $CC1_4$ (0.4mls/kg) on day 14.

Group F (prophylactic treatment group) received 200mg/kg daily for 2 weeks followed by a single dose of $CC1_4$ (0.4ml/kg) on 'day 14'.

3.2 Animals Sacrifice

At the end of the experiment (day 15), all the animals were humanely sacrificed. Blood was collected through the left ventricle of the heart of the animals in heparinize centrifuge tube under a deep anesthesia with chloroform. The blood collected was centrifuged using centrifuge machine at 10,000 rmp for five minutes and

the serum collected was subjected to liver function test (AST, ALT and ALP) and estimation of oxidative stress enzymes (SOD, CAT and MDA). The liver tissue was harvested for histological examination.

3.3 Biochemical Assay Liver Function Test

The liver enzymes analysis; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) was carried out using an auto-analyzer.

3.4 Estimation of Oxidative Stress Enzymes

The liver oxidative stress makers analysis were carried out for the following: superoxide dismutase (SOD), Lipid Peroxidation (malondialdehyde), Catalase (CAT) using auto- analyser.

3.5 Data Analysis

Results obtained were analysed using the statistical software, Statistical Package for Social Scientist (SPSS *version* 18.0) and results were expressed as mean ±SEM. Differences among means of the groups were determined using one way ANOVA with *LSD post hoc test*. Paired sample *t*-test was also used as appropriate and values were considered statistically significant when $p<0.05$.

4. Results

4.1 Physical Observation of Animals

During the period of administration of the aqueous extract of *Garcinia kola* seed, all the experimental animals (Wistar rats) were observed to have normal physical activity. Weight changes were observed in the experimental animals, day 1 before administration and day 15 after administration of the experiment, with their weight differences. During the period of administration, Group A had active physical activities, and ate more when compared to other group and on the day 14 after administration of carbon tetrachloride Group B had limited physical activities, writhing, gasping and a decrease in feeding when compared to the control group (Group A).

Table 1 shows body weight changes in experimental animals using one-way ANOVA. Weight changes were observed in the groups. Increase in body weights of animals were observed in group A (control), group C (Silymarin + $CC1_4$), group D (extract) and group F (extract low + $CC1_4$). However, results revealed that animals in groups B ($CC1_4$) and E (extract high + $CC1_4$) exhibited decrease in body weight.

There was significant decrease ($p<0.05$) in the body weight of group B animals when compared to control. However, results revealed significant increase ($p<0.05$) in body weights of animals in groups C, D, E and F when compared to $CC1_4$.

Table 1. Effect of *Garcinia kola* aqueous extract, silymarin and $CC1_4$ administration on the body weight of Wistar rats

Groups (n)	Initial weight (g)	Final weight (g)	Body weight difference (%)
Group A	63.30±10.47	100.00±11.32	33.70±11.52
Group B	66.84±15.75	55.56±16.34*	-11.28±11.43*
Group C	71.58±18.31	115.60±21.49**	44.03±4.83**
Group D	70.62±7.53	107.66±10.54**	37.04±5.01**
Group E	71.32±7.87	67.62±9.29	-3.70±2.03
Group F	70.34±16.06	107.20±10.57**	36.86±6.35**

Note: *,** $p<0.05$ when compared to negative control and experimental groups respectively.

4.2 Liver Enzymes: AST, ALT and ALP

Table 2 revealed that the CCL4 treated group (group B) expressed remarkably high levels of liver enzymes (Alanine Phosphatase, ALP and Alanine aminotransferase, ALT). There was decrease in the level of liver enzymes aspartate aminotransferase, AST. There was increase in ALP level at group B, D and F treatment when compared to control; however, this was not significant. Rats that received a single dose of CC14 (group B) showed marked elevation in the levels of liver enzymes when compared with that of the control (group A) rats. Pretreated groups C, D, E and F rats revealed significant increase in the levels of AST and decrease in ALT.

However, there was decrease in ALP level significant with Silymarin + extract low dose administration when compared with group B rats.

Table 2. Biochemical Assay of Liver Enzymes following oral administration of *Garcinia kola* seed aqueous

extract in $CC1_4$ induced rats

Groups	ALT (U/L)	ALP (U/L)	AST (U/L)
Group A	33.20±1.81	76.89±4.06	101.60±3.73
Group B	46.84±1.99*	82.26±3.97*	79.62±4.10*
Group C	40.59±1.69	77.49±3.85	99.37±1.30
Group D	36.36±3.83	87.39±4.67	83.40±7.76
Group E	40.59±2.09	77.69±3.94	99.58±1.50
Group F	39.68±3.22	80.14±2.81	101.77±3.45**

Note: *,** $p<0.05$ when compared to negative control and experimental groups respectively.

4.3 Oxidative Stress Indicator

Results depicted in Table 3 showed that administration of Garcinia kola seed aqueous extract caused insignificant increase in the levels of SOD as compared to the low levels observed in the CC14 treated group. However, there is also no significant difference in the increase of the catalase levels when compared to CC14 group. In the liver tissue, increased levels of lipid peroxides were recorded in the group B rats. The activities of the peroxides decreased with increase in the amount of the Garcinia kola seeds extract administered.

This is evidenced in the values obtained in rats in groups E and F.

Table 3. Serum levels of catalase (CAT), lipid peroxidation malonaldehyde (MDA) and superoxide dismutase (SOD) on the effect of *Garcinia kola seed aqueous extract* on oxidative stress markers in $CC1_4$ induced-hepatotoxity

GROUPS	SOD (U/mg pro)	CAT (U/mg pro)	MDA (U/mg pro)
Group A	29.55±1.97	18.91±1.81	0.66±0.11
Group B	15.41±1.91*	8.81±0.89*	3.65±0.51*
Group C	23.56±4.23**	9.50±0.66*	2.06±0.26
Group D	18.75±1.31	17.42±3.64**	1.08±0.11**
Group E	22.96±4.56**	16.76±1.57**	0.90±0.07**
Group F	18.17±2.06	17.10±3.37**	0.70±0.12**

Note: *,** $p<0.05$ when compared to negative control and experimental groups respectively.

4.4 Histopathological Studies of Liver

4.4.1 Histological Features of the Control (Untreated) Experimental Animals

Liver sections of control group showed normal histoarchitecture of the liver parenchyma; the characteristic appearance of the hepatic cells (hepatocytes) having preserved cytoplasm, prominent nuclei and nucleoli, and hepatocytes radiating from the central vein. Fine vascular separate the thin plate of hepatocytes, the sinusoids Liver sections of the $CC1_4$ treated group (group B) showed distortion in the histoarchitecture of the liver parenchyma, such as, areas of vacuolar degeneration, necrotic cells, and congestion (blood cells in the hepatocytes). Infiltration of inflammatory cells, around the blood vessels, was also observed (Plate 2). Liver sections of Wistar rats of the standard drug (silymarin) group (group C) showed normal liver histoarchitecture with few inflammatory cells seen (Plate3).

Sections of liver of the extract (*Garcinia kola seed*) in the treated group (extract control; group D) showed the histology of liver parenchyma comparable to that of the control group with normal hepatocytes with preserved cytoplasm, prominent nuclei, and nucleoli and central vein (Plate 4).

Administration of *Garcinia kola seed* extract preserved the histoarchitecture of the liver against $CC1_4$ administration, at low and high doses. Mild vacuolar degeneration was observed in the liver sections of Wistar rats treated with high dose extract (Plate 5). Comparatively, the low extract dose presented a better hepatoprotective effect on the histology of the liver (Figure 6).

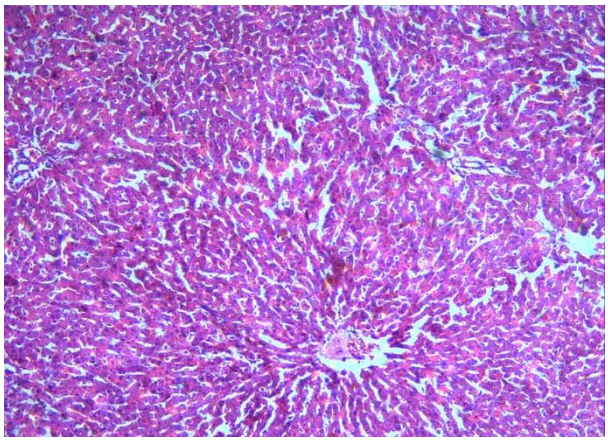

Figure 1. Liver section of group A rats showing normal liver histoarchitecture (H & E, x 400)

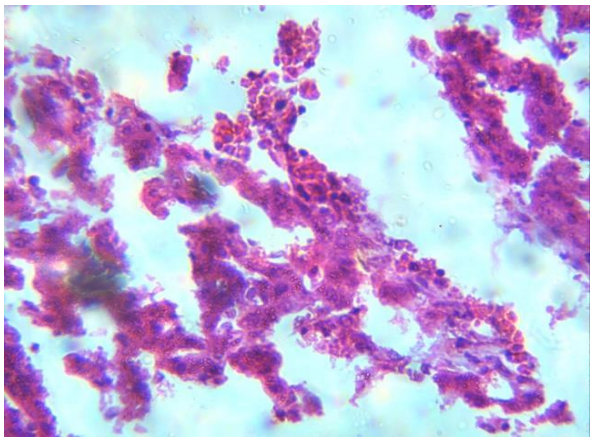

Figure 2. Liver section of $CC1_4$ treated group B showing distorted liver histoarchitecture; Congested central vein, congestion, necrosis of hepatocytes (H & E, x 400)

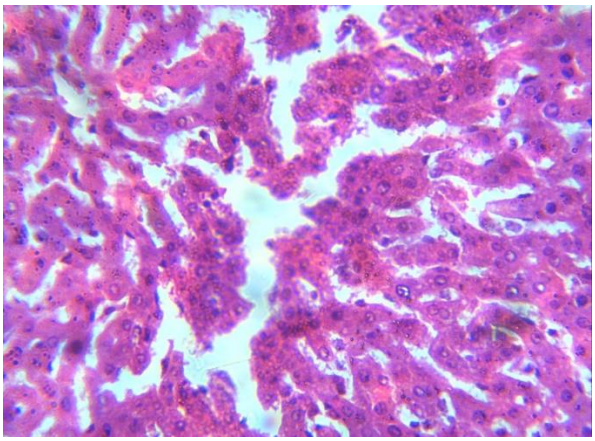

Figure 3. Liver section of Silymarin and $CC1_4$ treated group C showing mild distortion of the liver histoarchitecture; central vein, sinusoids with few necrotic cells (H & E, x 400)

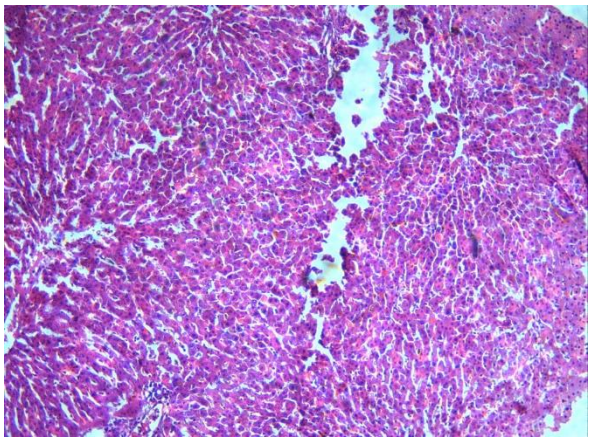

Figure 4. Liver section of extract (400mg/ kg) treated group D showing the liver histoarchitecture (H & E, x 400)

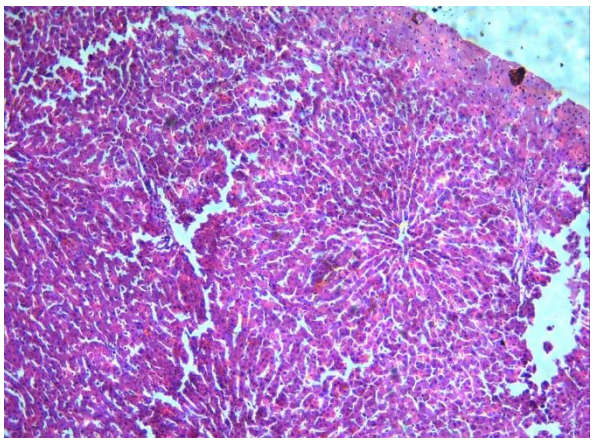

Figure 5. Liver section of extract & CC14 treated group showing mild distortion of the liver histoarchitecture and Infiltration of inflammatory cells

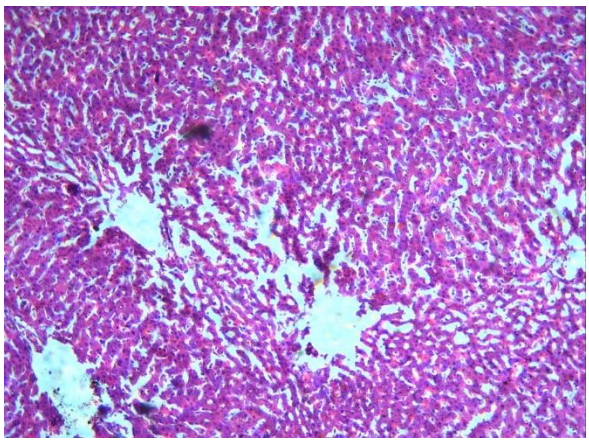

Figure 6. Liver section of extract & $CC1_4$ treated group showing preservation of the liver histoarchitecture

5. Discussion

Body weight fluctuations have been utilized as a sign of pharmacological and chemical side effects (Mukinda and Syce, 2007). The body weights of the experimental animals (Wistar rats) changed, as seen by physical examination. The CC14-treated group (group B) showed a significant fall in body weight, which may have been caused by the toxic effects of CC14 treatment. This supports earlier research on the toxicity of CC14 (Obi et al., 1998; Nevien, 2012).

Growth is what caused the group A (control) animals' weight to increase as seen. Animals treated with CC14 (group B) and high dosage extract plus CC14 (group E) had lower body weights, which may have been caused by treatment-related intoxication. This may possibly be due to the animals in these groups having reached adulthood, which would explain the weight loss. Increased body weights in the extract low + CC14 treated group (group F) have been observed, which may indicate that the extract has some partial potency against CC14 toxicity.

The hepatoprotective effect of plants against CC14-induced hepatotoxicity was documented in several research relating to herbal and traditional medicine (Prakesh et al., 2008; Biswas et al., 2010; Osman et al., 2011; Sahreen et al., 2011). This result is consistent with the study on the CC14-fighting ability of Garcinia kola extract (Adaramoye, 2010). The significant gain in body weight of the extract-high group (group D) demonstrates the safety of the extract at this level (400mg/kg) and that it has no effect on weight. Garcinia kola was shown to be non-toxic at doses larger than 900 mg/kg and to be deadly at 6741.43 mg/kg, according to reports on its acute toxicity (Duze et al., 2011; Udenze et al., 2012).

The observed rise in body weights of the group receiving silymarin + CC14 suggests that silymarin is a conventional hepatoprotective medication, providing protection (Prakesh et al., 2008). The delivery of CC14 caused considerable liver damage, as indicated by the rise in the levels of the liver marker enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP), and reduction in aspartate aminotransferase (AST). The increased values of these biochemical measures are unquestionably signs of changes in the structural and functional integrity of the liver (Obi et al., 1998; Sahreen et al., 2011).

The elevated levels of liver enzymes found in this study are consistent with earlier publications on the toxicity of CC14; toxicity in the liver brought on by CC14 and other chemicals is thought to be caused by the toxic metabolites produced (Arulkumaran, 2007; Gnanaprakash et al., 2010). According to Williamson et al. (1990) and Ojo et al. (2006), ALT is more specific to the liver and is hence a superior measure for identifying liver damage. The extraordinary (p 0.01) increase in ALT enzyme level seen with CC14 treatment in group B mice is suggestive of liver injury. Once the cellular membrane is compromised, these enzymes, which are housed in the cytoplasm of the cell, are released into the bloodstream (Iroanya et al., 2012).

Workers have come to the conclusion that when CC14 is reductively dechlorinated, a trichloromethyl radical is produced, which is how CC14 causes liver damage. A hydrogen atom from a fatty acid is drawn to the trichloromethyl radical by it, creating a lipid radical that interacts with molecular oxygen. The beginning of lipid peroxidation is the result of such a process. With the administration of silymarin, the level of ALP significantly decreased.

Usmani and Kushwaha (2010) observed that silymarin administration resulted in lower levels of ALP when compared to higher levels brought on by the administration of CC14. According to publications on the effects of the plant on the liver (Ferenci et al., 1989; Usmani and Kushwaha, 2010; Osman et al., 2011), silymarin may have had a hepatoprotective impact on the liver enzymes. Since the aforementioned method points to the existence of oxidative stress, it follows that any naturally occurring substance with antioxidant properties would stop or reverse lipid peroxidation, including damage to cell membranes (Ulicna et al., 2003; Osman et al., 2011).

The screening of Garcinia kola's natural antioxidant activities was influenced by the report of Iwu (1982), which mentioned the use of the plant's seeds in traditional medicine and herbal remedies for treating liver diseases. Another argument for its antioxidant status is that a comparison of the findings with those of silymarin, one of the most potent antioxidants, would be favorable. According to the results of this investigation, pretreatment of rats 14 days before CCL4 delivery resulted in a noticeably lower level of hepato-specific blood enzymes. This shows that Garcinia kola seeds may be able to protect rats' livers from CC14-induced liver damage. This was established by comparing the outcomes of rats that had previously received Garcinia kola treatment.

The degree of drug-induced liver damage can be predicted using antioxidant enzymes and lipid peroxidation levels (Ogunlade et al., 2012). In the metabolic processes that contain free radicals, antioxidant enzymes like as superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) work in a dependent manner. Indicators of liver damage include changes in the activity of antioxidant enzymes and the oxidative stress markers CAT, SOD, and MDA (Sahreen et al., 2011; Nevein, 2012). By peroxiding unsaturated fatty acids, which also changes the ratio of poly-unsaturated to other fatty acids, extensive lipid peroxidation causes membrane disorder. As a result, the fluidity of the membrane would be reduced, and the cell would eventually

die.

The toxic action of CCL4 is responsible for the rise in MDA, decrease in CAT, and increase in SOD. The CC14-treated group (group B) showed decreased antioxidant enzyme activity (CAT, SOD), as well as elevated antioxidant enzyme activity (MDA), which is indicative of treatment-related toxicity. This is consistent with findings from research on the toxicity of CC14; CAT, SOD levels that are decreasing and MDA levels that are increasing in the liver imply that CC14 has a toxic effect on the liver (Kumar and Kumar, 2012).

Silymarin is a powerful hepatoprotective agent, according to results. According to the data (Table 3), the extract at high dose exhibits superior hepatoprotection than the extract at high dose, as evidenced by liver enzyme levels and oxidative stress markers. According to Gnanaprakash et al. (2010), a histological test can be utilized to demonstrate the extent of the liver damage caused by CC14. The CC14-induced group (B)'s severely altered hepatocyte arrangement, which includes regions of inflammatory cells, necrosis, a clogged central vein, inflammatory cell infiltration, and vacuolar degeneration, is an example of histoarchitectural distortion (Plate II).

These are all symptoms of the toxicity brought on by CC14. According to several researches, CC14 treatment causes histological alterations such necrosis (Kumar & Kumar, 2012; Nevien, 2012). Mild In contrast to the control group, the silymarin group (100 mg/kg) showed minor necrosis and the presence of inflammatory cells as a result of the hepatocytes' histoarchitectural deformation. A pathological form of death called necrosis happens in response to aberrant stimuli such chemical damage or toxin exposure. Inability to retain membrane integrity causes necrotic cells to bleed out their contents, which can cause inflammation in the surrounding tissue (Kumar et al., 2009).

Inflammation is basically a defensive reaction, and its ultimate purpose is to rid the body of both the toxins that caused the cell damage in the first place as well as the effects of that damage, such as necrotic cells (Kumar et al., 2009). Silymarin, a common medication, was administered at a dose of 100 mg/kg to protect the liver cells from the harmful effects of the toxic chemical (CC14). While there was some minor necrosis seen in a few places in this group's histoarchitecture, the deformation was not as severe as it was in the control group. This may be because to the medication's hepatoprotective properties. After the administration of the aqueous extract of Garcinia kola seed, there was a discernible improvement in the microscopic appearance of the liver, showing restoration in the hepatocytes, mild cytoplasmic congestion, and absence of centrilobular necrosis with a nearly visible central vein (groups E and F). However, compared to the control group, the distortion was less severe, particularly in the 200mg/kg group. The extract treatment's hepatoprotective action may have caused the sparing restoration of the hepatocytes in these groups.

An attempt is made to recreate the injured tissue as a result of a series of processes that are set in motion by the cellular response to cell injury, which acts to eliminate, dilute, or block off harmful chemicals (Kumar et al., 2009). Histoarchitectural preservation or protection of the hepatocytes was more successful at a dosage of 200mg/kg in the groups who received Garcinia kola seed. The biochemical activity studies, as shown in Table 2, are supported by this finding. Patients with liver problems might rely on this naturally occurring plant as a supplement therapy since Garcinia kola is thought to have antioxidant components (Adaramoye, 2005) that can mitigate the harm caused by CC14. These results suggest that Garcinia kola seed could be able to reduce the effects of CC14 on the rat liver.

6. Conclusion

The results of this study indicate that the aqueous extract of Garcinia kola seed seeds may effectively prevent CC14-induced liver damage and protect the liver from oxidative damage.

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