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Hepatoprotective Effect of Ethanolic Extract of *Cnestis ferruginea* Roots on Carbon tetrachloride-Induced Liver Damage in Male Rats

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ABSTRACT: Hepatoprotection or antihepatotoxicity is the ability of an agent or chemical to prevent damage to the liver. These hepatoprotective agents mitigate the liver injury caused by the hepatotoxic agents. Therefore, the objective of this paper was to investigate the hepatoprotective effect of ethanolic extract of *Cnestis ferruginea* roots on carbon tetrachloride (CCl4)-induced liver damage in male rats using appropriate standard methods. A single dose of CCl_4 administration significantly ($p<0.05$) increased the levels of ALT and AST in the positive control group, compared to the negative control group. Also, a significant ($p<0.05$) increase in the level of ALP and TB was seen in the positive control group relative to the negative control group. While, pre-treatment with either the extract or the standard drug significantly ($p<0.05$) reduced the levels of hepatic enzymes of serum (ALT, AST and ALP) and TB, when compared with the positive control group. Similarly, the activity of Glutathione-s-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the liver of untreated rats (induced with CCl_4)was significantly decreased ($p<0.05$) in comparison with the rats treated with the extracts and the standard drug. Thus, treatment of the CCl_4 -induced rats with the extracts for fourteen days resulted in the reversal of the CCl_4-induced liver damage in the rats with the high dose (200 mg/kg) giving the best result which was comparable with the standard drug. Therefore the ethanolic extract of *C. ferruginea* roots protects the liver against CCl_4 -induced oxidative stress.

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Continuous use of synthetic medicines is associated with a high prevalence of liver damage in patients, as reported by Singh *et al*. (2021). This phenomenon may occur due to the hepatic metabolism of a majority of medicines, leading to potential impairment of liver function. The utilization of natural materials presents itself as a viable approach to addressing these issues. Plants of several classifications have historically been employed in the practice of traditional medicine. Presently, a multitude of investigations employs plant samples to ascertain the efficacy of bioactive compounds that hold potential as prospective pharmaceutical agents in subsequent medication development endeavours (Saha *et al.*, 2019). *Cnestis ferruginea* Vahl ex DC is a perennial shrub that exhibits a wide distribution over the savannah region of tropical West Africa (Atere and Ajao, 2009). *C. ferruginea* (Connaraceae), known as Fura amarya and otito among the Hausa tribe in northern Nigeria, Okpu nkita and "amunkita" among the Igbo tribe in southeast Nigeria, and Akara oje and Bonyin bonyin among the Yoruba tribe in Southwest Nigeria, is a climbing plant found in deciduous forests (Burkill, 1985). The roots of *C. ferruginea* have been traditionally employed for

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several medicinal purposes, including their usage as a laxative, aphrodisiac, treatment for ovarian diseases, medicine for abortion complications, and as a therapeutic agent for certain skin infections, sore throat, migraines, and sinusitis (Ha, 2017). The plant exhibits several biological and pharmacological activities, including anti-inflammatory and anti-nociceptive properties (Ishola *et al*., 2011), antioxidant effects (Ita, 2017), hypoglycemic effects (Adisa *et al.,* 2011) and antibacterial effects (Acheampong *et al.*, 2018). Additionally, it has been studied for its hepatoprotective effect and analgesic effects, as well as its anti-stress properties (Akharaiyi *et al.,* 2012; Ishola and Ashorobi, 2007). Also, Ishola and Ashorobi (2007) found the median lethal dose (LD_{50}) to be 3.6570 g/kg. The wound management property of hydroethanolic leaf extract of *C. ferruginea* has also been stated (Yakubu *et al.,* 2021). Also, the protection of CCl_4-Induced Liver and Kidney Damage by Phenolic Compounds in Leaf Extracts of *C. ferruginea* has been reported (Rahmat *et al.,* 2014). Although the leaf and roots of *C. ferruginea* are used in folklore medicine for wound healing and other varied diseases, there is a paucity of information on the liver protective effects of its root extract. Therefore, the objective of this paper was to investigate the hepatoprotective effect of ethanolic extract of *Cnestis ferruginea* roots on carbon tetrachloride (CCl4)-induced liver damage in male rats.

MATERIALS AND METHODS

Drug and Chemicals: Silymarin and Carbon tetrachloride (CCl4) were purchased from Sigma chemicals, USA. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phosphatase (ALP), Serum Glutamat Pyruvate Transaminase (SGPT), Serum Total bilirubin (T.Bil), Albumin, Total protein, Cholesterol (CHOL), HDL- Cholesterol (HDL), Triglycerides (TRIGS) kits were purchased from RANDOX Laboratories Ltd., Ardmore, UK. All other chemicals for this study were of analytical grade.

Collection and Preparation of Plant Materials: Cnestis ferruginea (roots), were collected from a farmland at Abraka in Delta State and authenticated at the Department of Plant Biology and Biotechnology, University of Benin (with voucher No. UBH-C369). They were prepared and extracted as described by Okoro (2020a) and the extract was kept at 4 °C before use. Thereafter, the dried extract were suspended in distilled water to prepare the two doses (200, and 100 mg/ kg b.wt) used in this study.

Animals: The animals (weighing 150 - 230 g) used for the study were bought from the Anatomy Department of Delta State University, Abraka, Nigeria. They were fed grower's mash (Top Feed, Ltd, from Sapele in Delta State) and water *ad libitum* and maintained in agreement with the guidelines on the care and wellbeing of research animals (Olfert *et al.,* 1993).

Grouping and Treatment of Animals: The male albino rats used for the study were grouped into five (groups A- E) groups of five rats each and were treated orally for 14 days as follows:

Group A- Animals + distilled water only (negative control).

Group B- Animals + CCl_4 in olive oil vehicle only (positive control).

Group C- Animals + 200 mg/ kg-day extract.

Group D- Animals + 100 mg/ kg-day extract.

Group E- Animals + 100 mg/kg bw of silymarin.

Treatment of the rats continued for 14 days and groups B – E animals were injected intraperitoneally with CCl_4 in an olive oil vehicle at a dosage of (1 ml/kg bw) on the 14th day (30 min after the last treatment). The animals were fasted and sacrificed by cardiac puncture, 24 h after CCl_4 administration to obtain the serum and the liver tissue used for the assays of this study.

Serum Biochemical Parameters Determination: Serum samples were used for hepatic function tests by assaying for Albumin (ALB), Total bilirubin (TB), Total protein (TP), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities using standard diagnostic kits (from RANDOX Laboratories Ltd., Ardmore, UK

Oxidative Stress Markers Determination: The following methods were used for the assays: The levels of antioxidants in liver tissue: SOD, CAT, Glutathione (GSH), GPx, and Glutathione-s-transferase (GST), were estimated by the method of Marklund (1992), Sinha (1972), Ellman (1970), Rotruck *et al*. (1973) and Habig *et al*. (1974). While Lipid peroxidation byproduct [malondialdehyde (MDA)] in liver tissue was by Ohkawa *et al*. (1979).

Statistical analysis: Data was analysed by Prism Graphpad software, version 6.0. The statistical difference between groups was evaluated by the one-way ANOVA, followed by Turky's post hoc test. The significance level was set at $P < 0.05$. While results were given as the mean ± SD.

RESULTS AND DISCUSSION

Biochemical changes in serum parameters: The impact of ethanolic extracts derived from *C. ferruginea* on the hepatic functionality of rats subjected to CCl_4 intoxication is illustrated in Figures 1-6, following a treatment period of 14 days.

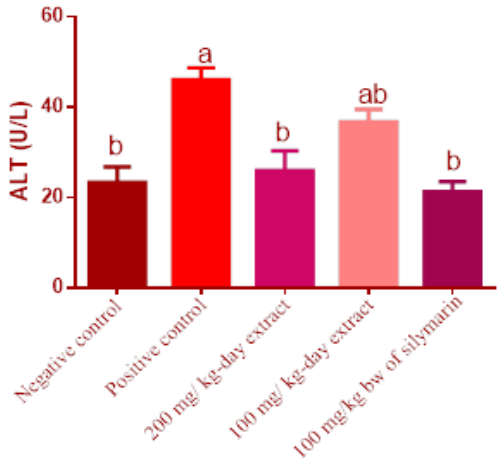

Fig 1: Effects of ethanolic extracts of *C. ferruginea* and silymarin on alanine aminotransferase (ALT) activity
a p(0.05) vs negative control; b (p<0.05) vs positive control

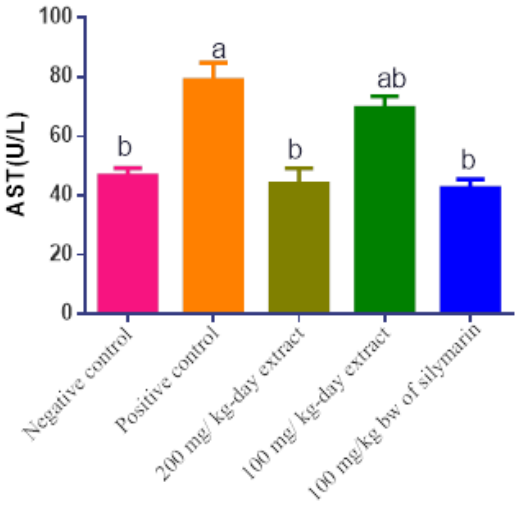

Fig 2: Effects of ethanolic extracts of *C. ferruginea* and silymarin on aspartate aminotransferase (AST) activity
a p(0.05) vs negative control; b (p<0.05) vs positive control

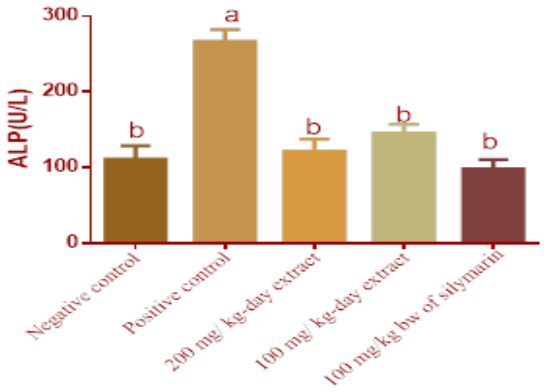

Fig 3: Effects of ethanolic extracts of *C. ferruginea* and silymarin on alkaline phosphatase (ALP) activity
a p(0.05) vs negative control; b (p<0.05) vs positive control

The delivery of a single dose of CCl_4 resulted in a statistically significant rise ($p<0.05$) in the levels of ALT and AST in the positive control group, as compared to the negative control group. Furthermore, a statistically significant rise ($p<0.05$) in the levels of ALP and TB was seen in the positive control group of rats compared to the negative control group. In contrast, prior administration of either the extract or the standard drug resulted in a substantial decrease ($p<0.05$) in the serum levels of hepatic enzymes (ALT, AST, and ALP) and TB, as compared to the positive control group. In contrast to the negative control group of rats, the positive control group exhibited a decrease in albumin and total protein levels. The rats administered either the greater dose of extract or the conventional medication (silymarin) had considerably ($p<0.05$) elevated levels of these two parameters.

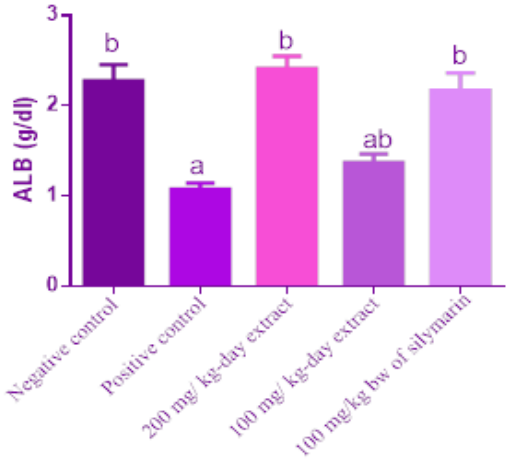

Fig 4: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Albumin (ALB) level
a p(0.05) vs negative control; b (p<0.05) vs positive control

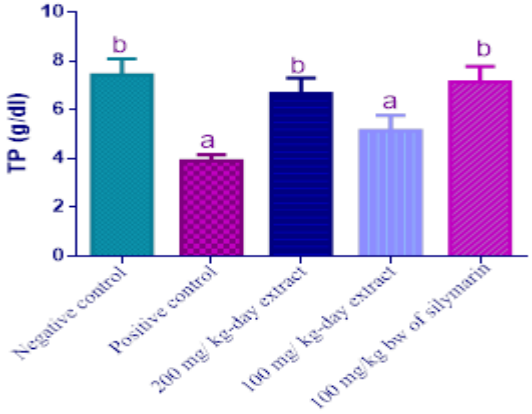

Fig 5: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Total protein (TP) level
a p(0.05) vs negative control; b (p<0.05) vs positive control

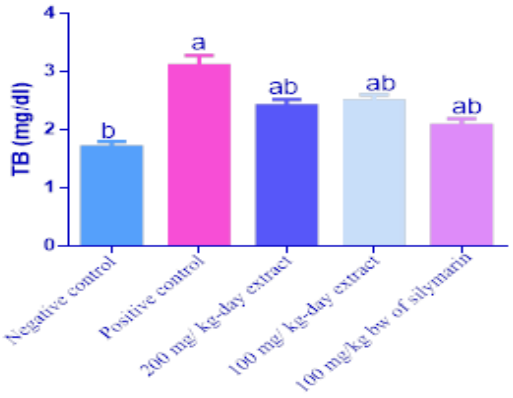

Fig 6: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Total bilirubin (TB) level
a p(0.05) vs negative control; b (p<0.05) vs positive control

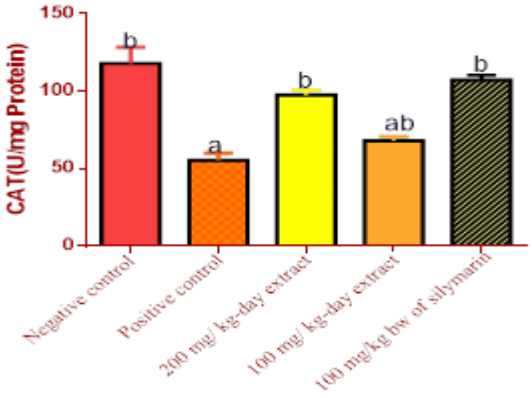

Fig 8: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Catalase (CAT) level
a = p(0.05) vs negative control; b = (p<0.05) vs positive control

Superoxide dismutase (SOD) and Catalase (CAT) activities: The study observed a substantial decrease in the activity of superoxide dismutase (SOD) in liver tissue homogenate of animals treated with CCl_4, as compared to the negative control group. Nevertheless, the administration of *C. ferruginea* extract with silymarin resulted in a noteworthy augmentation of superoxide dismutase (SOD) activity across all experimental groups (Figure 7). In comparison to the negative control group, the CAT activity in rats treated with CCl_4 was considerably reduced ($p< 0.05$), specifically in the positive control group. Once more, the application of pretreatment with extract resulted in a substantial restoration of catalase (CAT) activity within the groups that were treated with the extract, as depicted in Figure 8. In a similar vein, administration of the silymarin resulted in a considerable augmentation of catalase activity, as depicted in Figure 8).

Lipid Peroxidation (LPO) and Glutathione (GSH) levels: The impact of C. ferruginea extracts on lipid peroxidation (LPO) was assessed by quantifying the production of malondialdehyde (MDA) in liver tissue after exposure to carbon tetrachloride (CCl_4), as depicted in Figure 9. Following the treatment of CCL_4, there was a notable and statistically significant rise ($p< 0.05$) in the levels of MDA seen in the rats assigned to the positive control group. Nevertheless, administration of the high dose (200 mg/kg) extract, in conjunction with silymarin, exhibited a substantial suppression of MDA production within the experimental groups. In contrast, a statistically significant drop ($p <0.05$) in the concentration of glutathione (GSH) was seen in rats treated with CCL_4 (positive control) as compared to the negative control group. The administration of the ethanolic extract of the plant for 14 consecutive days resulted in a significant protective effect against the depletion of GSH in the groups that received the treatment (Figure 10).

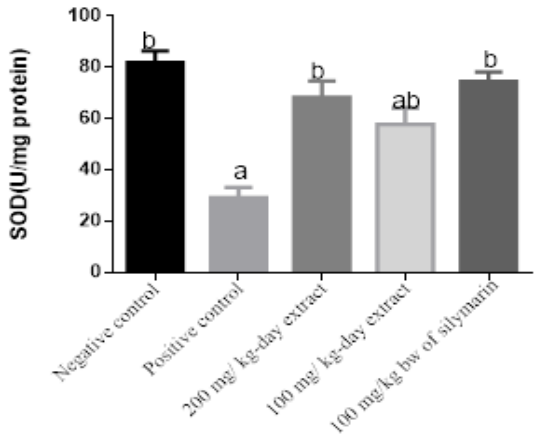

Fig 7: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Superoxide dismutase (SOD) level a *a = p(0.05) vs negative control; b = (p<0.05) vs positive control*

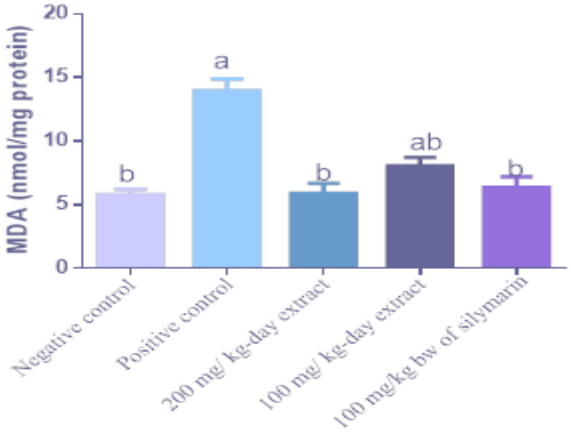

Fig 9: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Malondialdehyde (MDA) level a *a = p(0.05) vs negative control; b = (p<0.05) vs positive control*

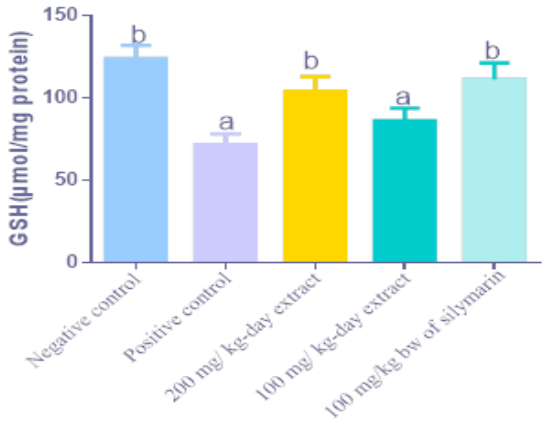

Fig 10: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Glutathione (GSH) level
a = p(0.05) vs negative control; b = (p<0.05) vs positive control

Glutathione Peroxidase (GPx) and Glutathione-S-transferase (GST) Activities: Figure 11 illustrates the observed levels of Glutathione Peroxidase activity in the liver samples. A statistically significant decrease ($p<0.05$) was seen in the rats treated with CCL_4, whereas the administration of the plant's extract resulted in a statistically significant rise ($p<0.05$) in GPx compared to the CCL_4-treated group. Likewise, a statistically significant increase ($p<0.05$) was detected in the groups treated with silymarin when compared to the positive control group. In a similar vein, the activities of GST were shown to be considerably reduced ($p<0.05$) in rats treated with CCl_4, in comparison to rats in the negative control group. However, the administration of the extract at both doses exhibited the ability to restore the GST activity to levels comparable to those observed in the normal condition (Figure 12). Additionally, the rats treated with silymarin displayed a pattern that was comparable to the animals treated with a the high dose of extract.

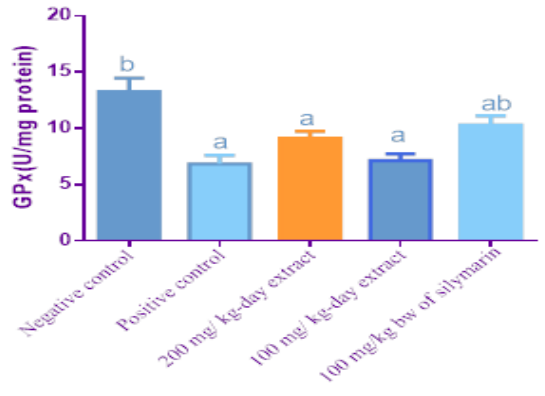

Fig 11: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Glutathione peroxidase (GPx) activity *a p(0.05) vs negative control; b (p<0.05) vs positive control*

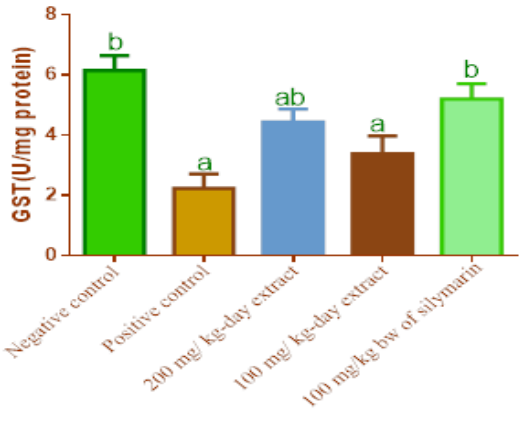

Fig 12: Effects of ethanolic extracts of *C. ferruginea* and silymarin on Glutathione-s-transferase (GST) activity
a p(0.05) vs negative control; b (p<0.05) vs positive control

The roots of *C. ferruginea* have historically been utilized for various medicinal applications, encompassing their utilization as a laxative, aphrodisiac, remedy for ovarian diseases, treatment for complications arising from abortions, and as a therapeutic agent for specific skin infections, sore throat, migraines, and sinusitis (Ha, 2017). The plant demonstrates various biological and pharmacological activities, such as anti-inflammatory and anti-nociceptive properties (Ishola *et al*., 2011), antioxidant effects (Okoro, 2023; Ita, 2017), hypoglycemic effects (Adisa *et al*., 2011) and antibacterial effects (Acheampong *et al*., 2018). Furthermore, extensive research has been conducted to investigate the hepatoprotective, analgesic and anti-stress capabilities of the plant. While the traditional usage of *C. ferruginea*'s leaf and roots in folk medicine for wound healing and various ailments is known, there is a lack of research on the hepatoprotective benefits of its root extract. Consequently, the primary objective of this work was to assess the hepatoprotective properties of the ethanolic extract derived from it in rats treated with CCl_4.

In this study, the induction of rats with CCL_4 significantly increased the serum activity of AST and ALT which agrees with the reports of Mansouri *et al*. (2017) and Karale and Kamath (2017). However, pretreatment with *C. ferruginea* extract significantly prevented the CCl_4- induced hepatic injury by causing a decrease in the serum activity of ALT and AST. It has been previously reported that CCL_4 can cause hepatic damage (El-Sayed *et al*., 2015; Ponmari*et al*., 2014; El-Gazayerly*et al*., 2014). Liver marker enzymes such as AST and ALT escape into the bloodstream as a result of hepatocellular damage which could be necrosis, loss of hepatic architecture,

inflammation or degeneration of hepatocytes. These enzymes are mostly used as indicators of hepatocellular necrosis (Okoro *et al.,* 2022; Dama *et al*., 2011). The significant increase observed in the serum activities of AST and ALT in the positive control rats could be due to hepatocellular damage since they are usually found in the cytoplasm and only released into circulation after cellular damage (Hassan and El-Gendy, 2003). These results agree with the findings of Naziroglu*etal* (1999) and Ahmed *et al*. (2000) who observed similar effects on these markers enzymes after induction with CCL_4. In this study, the activity of Glutathione-s-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the liver of untreated rats (induced with CCl_4) were significantly decreased in comparison with the rats treated with the extracts and the standard drug, while the level of MDA was drastically increased, which is in agreement with previous studies (Sharma and Shukla, 2011; Asagba *et al.*, 2010; Park *et al*., 2010). But pretreatment of the animals with both doses of the extract prevented the CCl_4-induced decrease in GST, SOD, CAT and GPx, which was evident in an increase in the levels of these oxidative stress makers to a comparable level with the standard drug dose-dependently. Thus confirming the antioxidant and anti-stress properties of *C. ferruginea* extract (Okoro *et al.,* 2019; Ita, 2017; Ishola and Ashorobi, 2007). In this experiment, lower levels of albumin and total protein were noticed in the positive control rats, compared with the negative control group of rats. While significantly higher levels of these two parameters were seen in the rats given either the higher dose of extract or the standard drug. However, an elevation in the level of total serum bilirubin was observed in the CCL_4 hepatotoxic rats, indicating an abnormal conjugation of bilirubin by the liver due to hepatic cell damage (El-sherbiny*et al.,* 2003). However, after treatment with ethanolic extract of *C. ferruginea* and silymarin, there was a decrease in the level of total serum bilirubin in CCL_4-induced rats. This agrees with the report of Feher *et al*. (1987) who reported that silymarin can reduce the elevated level of total bilirubin caused by hepatic injury. For *C. ferruginea*, this could be due to its antioxidant activity (Okoro, 2020b; Ita, 2017). Results of this study showed that the induction of rats with the CCl_4 resulted in the depletion of antioxidant enzymes. The attack of polyunsaturated fatty acids by reactive oxygen species (ROS) which culminates in membrane structural and functional damage is a result of lipid peroxidation. In liver fibrosis induced by CCL_4, lipid peroxidation markers are high (Okoro *et al.,* 2015; Al-Sayed *et al*., 2014). Lipid peroxidation is usually assayed using the marker MDA (Thanh, 2015). Thus, in this study, the administration of CCl_4 led to increasing levels of MDA in the positive control rats. However, treatment with the high dose of the plant extract and the standard drug significantly inhibited the formation of MDA in the treated groups, thereby corroborating the liver protective effects of ethanolic extract of *C. ferruginea* against CCl_4- induced oxidative stress.

Conclusion: C. ferruginea has a significant protective effect against CCl_4-induced hepatotoxicity. The ethanolic extract of *C. ferruginea* protects the liver against CCl_4 oxidative stress with the high dose of 200 mg/kg giving the best result which was comparable with the standard drug. The hepatoprotective effect exhibited by the extract may be through amelioration of lipid peroxidation by its scavenging activity of free radicals and enhancement of the antioxidant defence systems.

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