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ORIGINAL ARTICLE

Evaluation of Hepatoprotective Activity of *Dolichandrone atrovirens* Leaves Stem Extract against CCl_4-Induced Hepatic Damage in Wistar Rats

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Background: Exposure to environmental contaminants, including carbon tetrachloride (CCl4), induces hepatic damage. Certain extracts from Dolichandrone atrovirens (Bignoniaceae) protect against such damage.
Results: This research examines the preventive effects of hydroalcoholic extracts from the leaves of *D. atrovirens* (HE-DA) versus liver damage produced by CCl4 in rats. HE-DA was delivered orally to rats at three dosages (100, 200, and 300 mg/kg) in conjunction with CCl_4 (1 mL/kg in olive oil) for three weeks. Lipid profile indices, peroxidation levels, and antioxidant activity were assessed in rats' liver tissue. TC, TG, PL, FFA, and LDL levels were reduced. Hepatic malondialdehyde concentrations were decreased, and antioxidant activities were modified in rats treated with HE-DA. Histopathological analysis of the liver revealed that HE-DA therapy decreased fatty degeneration, cytoplasmic vacuolisation, and necrosis.
Conclusion: HE-DA had a protective effect against CCl_4-induced hepatotoxicity in rats, whose antioxidant capabilities may have mediated this effect.

Key words: Dolichandrone atrovirens; CCl4; Hepatoprotective; Lipid Profile, Antioxidants

Dolichandrone atrovirens (DA) (Bignoniaceae) is a deciduous plant distributed throughout India. Indigenous tribes and traditional healers traditionally used it to treat rheumatism, arthritis, diabetes, inflammation, and hepatic problems.(JU *et al*., 2019). The seeds that are part of *Dolichandrone atrovirens* possess diuretic and antispasmodic effects. The powder is used externally to reduce swelling, whilst a decoction of the bark is utilised for gastrointestinal pain; it also has antioxidant and antidiabetic attributes. Acknowledging that only a limited number of phytochemicals may exhibit therapeutic activity among the substances present in whole medicinal plants is crucial. The synthesis of phytochemicals depends on the particular parts of a plant (including bark, flowers, leaves, roots, fruit, or seeds) and the methods employed for their extraction. (Kavimani *et al*., 2014; Kayarohanam & Kavimani, 2015).

The liver is an essential organ that governs several critical biochemical and biological functions, such as homeostasis, growth, energy metabolism, nutrition transport, medicine and xenobiotic utilisation, elimination, and recovery from infection (Suchy, 2021). It is very susceptible to damage from hepatotoxic agents. Liver illness, or hepatic illness, is defined by compromised liver function leading to sickness. The liver executes several vital physiological activities, and the emergence of the disease may hinder these processes, compromising overall body function (Vicidomini *et al*., 2024).

Liver diseases are progressively acknowledged as a worldwide health concern, as per the WHO (Devarbhavi *et al*., 2023). The rate of death for acute liver illness in India is from 5% to 6.3%; for chronic liver conditions, including cirrhosis due to hepatitis B virus, it ranges from 17.6% to 47.9%, and for liver cancer associated with HBV, it varies between 40% and 60% (Yin *et al*., 2024).

Paracetamol, an effective painkiller and antipyretic with little side effects at typical therapeutic doses, may cause acute liver damage when used in high quantities with other narcotics or alcohol. (Freo *et al*., 2021). As a result, significant central medullary liver necrosis, which causes renal failure and perhaps death in humans and laboratory animals, may ensue. Herbal drugs are considered safe and devoid of significant adverse reactions, leading to a marked increase in their application for illness treatment worldwide (Chidiac *et al*., 2023). They can also be rapidly and easily acquired from nature. This study investigates the hepatoprotective efficacy of the hydroalcoholic solution of *Dolichandrone atrovirens* (DA) leaf by various in vivo methodologies.

MATERIALS AND METHODS

Sample Collection

DA leaves were collected from the Tirunelveli forest area (Tamil Nadu, India) and reported to the Botanical Survey of India (BSI) alongside a voucher specimen K.S.001. The specimens were verified by the Director, Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Family: Bignoniaceae.

Hydroalcoholic Extraction

Fifty grammes of powdered DA leaves were subjected to an automatic ultrasonic bath with a 1:10 ratio of 25% hydroalcoholic solution at 45 ± 1 °C. The beaker was capped with aluminium foil to minimise ethanol evaporation. The resulting solution was passed through filters, the residue was combined with a designated 25% hydroalcoholic solvent quantity, and the process was carried out until the extracts of hydro alcohol were cleared.

Experiment of Carbon tetrachloride-induced hepatotoxicity

Thirty-six Wistar rats that were albino were divided into six groups, each including six rats. The treatment sample and the reference medication were administered orally for 21 days. Group I Control (vehicle): Administered 0.9% saline at a maximum of 25 mL/kg body weight. Group II (CCl_4 induced): Administered CCl_4 and olive oil in a 1:1 v/v ratio, up to 0.5 mL/kg of body weight, by intraperitoneal injection. Group III (low dose of hydroalcoholic extract of DA (HE-DA)): Administered CCl_4 in conjunction with HE-DA at a 100 mg/kg dosage. Group IV (moderate dosage of HE-DA): Administered CCl_4 + 25% HE-DA at a maximum of 200 mg/kg. Group

V (elevated dosage of HE-DA): Administered CCl_4 with 25% HE-DA at a maximum of 300 mg/kg. Group VI (control group): Administered CCl_4 and Silymarin at 500 mg/kg.

On the 20th day, a dosage of 0.5 mL/kg of CCl_4 in olive oil (1:1 v/v) was delivered through the abdomen (i.p.) to groups II to VI after one hour of dosing with the conventional medication and hydroalcoholic extracts, and whereas group I received just 10 mL/kg of olive oil (i.p.). Following 24 hours, blood samples were obtained under moderate anaesthesia, after which all animals were euthanised by dislocation of the cervical spine, and the liver was extracted for biochemical examination. The body weight of the rats in every group was documented on the initial and 22nd days, which facilitated the calculation of weight change attributable to the therapy. The liver weight was measured to assess the drug's impact on the morphology and physiology of the rats.

Liver tissue homogenisation

The tissue homogenate underwent cold centrifugation at four °C (10,000× g for 15 minutes). The resulting liquid was gathered in tubes from the centrifuge and stored in a refrigerator at −80 °C till further examination (Barakat & Almundarij, 2020).

Biochemical Analysis

Determination of body weight and Liver weight

The fundamental tabletop balance was used to ascertain the body weight and liver weight of the experimental rats. Following the research period, the animals' body weights were assessed before and after the administration of CCl_4. (Ouassou *et al*., 2021).

Estimation of Lipid

Total cholesterol was assessed using the Zak technique (Zlatkis *et al*., 1953), triglycerides (Foster & Dunn, 1973), free fatty acids (Falholt *et al*., 1973), phospholipids (Bartlett, 1959), and high-density lipoprotein cholesterol. Friedewald *et al*. (1972) assessed very low-density lipoprotein and low-density lipoprotein cholesterol (Friedewald *et al*., 1972).

Determination of antioxidant markers

The liver homogenates underwent centrifugation at 5000 rpm for 10 minutes at 4°C. The resultant supernatant was used for the quantification of SOD. (Misra & Fridovich, 1972), GR (Moron *et al*., 1979), GST (Mannervik, 1985), CAT (Mahely & Chance, 1954), LPO (Ohkawa *et al*., 1979), Gpx (Rotruck *et al*., 1973), and GSH (Giustarini *et al*., 2013) Using a colourimetric technique.

Histopathological Analysis

Liver tissues were excised, washed in PBS at pH 7.4, and divided into two segments. A segment was designated for histological analysis (10% formalin), while the other 1 g segment was homogenised with 9 mL of PBS at pH 7.4 for in vivo evaluation. (Azab, 2014).

Statistical Analysis

All *in vivo* data are shown as the mean ± SEM (n = 6) and were analysed using a t-test followed by ANOVA. The significance levels are shown as * $p < 0.05$, compared to Group II.

RESULTS AND DISCUSSION

This research aimed to assess the hepatoprotective impact of HE-DA in rats treated with Carbon tetrachloride (CCl_4). The CCl_4 model of hepatotoxicity is thoroughly examined. It simulates oxidative stress in several pathophysiological contexts. Carbon tetrachloride, a recognised hepatotoxin, is a straightforward chemical that induces centrilobular hepatic necrosis and fatty liver in several species upon administration (Weber *et al*., 2003). It is a lipophilic molecule and is thus extensively dispersed throughout the body. Regardless of the delivery method, its primary harmful impact is on the liver (Clawson, 1989). Previous investigations on CCl_4 poisoning have shown that CCl_4 induces free radical formation in several organs, including the liver, kidney, brain, heart, lung, and blood. (Zimmerman & Lewis, 1995). The cytochrome P450 enzymes convert CCl_4 into the trichloromethyl (CCl_3) radical, a hepatotoxic metabolite. The covalent attachment of this radical to proteins triggers a series of events that progress from liver dysfunction to cellular necrosis (Teschke, 2018).

Hepatotoxins, including ethanol, acetaminophen, and CCl_4, cause liver damage, defined by variable degrees of hepatocyte degeneration and cellular apoptosis (Neuman, 2020). Evidence indicates that CCl_4 is frequently employed as a hepatotoxin in animal hepatopathy. The covalent binding of CCl_4 compounds, including trichloromethyl-free radicals, to proteins in cells is considered the first step in a series of events leading to membrane lipid oxidation and cellular death.

The average body weight of six experimental groups at 0 days (initial) and 21 days (final) is shown in Figure 1(A). The starting weight of the animals ranged from 140g to 150g. No substantial differences in initial weight were noted among the groups I (normal control), II (CCl_4 treated), III (CCl_4 treated with 100 mg/bw of HE-DA), IV (CCl_4 treated with 200 mg/bw of HE-DA), V (CCl_4 treated with 300 mg/bw of HE-DA), and VI (CCl_4 treated with 50 mg/Kg BW of Silymarin). Nonetheless, the ultimate body weight of experimental group II (treated just with CC14) exhibited a downward tendency. It was markedly different ($p < 0.05$) from the other experimental groups (I, III, IV, V, and VI).

The variations in body weight seen in Group II (CCl_4 treated alone) are attributable to CCl_4 induction, resulting from both the direct toxicity of CCl_4 and the indirect toxicity associated with hepatic injury. Alterations in body weight after CCl_4 administration have been used to indicate significant CCl_4-associated organ damage (Hussein & Khan, 2022). The ultimate body weight in group II (rats administered CC14) was substantially reduced compared to the control, HE-DA-treated, and standard drug-treated groups (Figure 1(A)).

The relative tissue weights of the liver were assessed in all groups (Figure 1(B)). Group I (7.53±0.15g), Group V (CCl_4 administered with HE-DA treatment, 6.09±0.13g), and Group VI (CCl_4 administered with standard drug treatment, 7.24±0.18g) exhibited no significant variation in liver weights. In contrast, Group II (3.86±0.11g) demonstrated a significant increase ($p < 0.05$) in weight compared to Groups I, V, and VI.

The liver weights of Group II exhibited substantial differences compared to Groups I, V, and VI. This investigation demonstrated that a 3-week regimen of discontinuous CCl_4 delivery led to a considerable rise ($p<0.05$) in liver wet weights (Figure 1(B)). The increase in the weight of the liver in the CCl_4 group is likely due to the accumulation of fat vessels observed by haematoxylin and eosin staining, together with elevated liver cholesterol and triglyceride levels.(Khalaf *et al*., 2009). Layman *et al*., (2019) observed a significant increase in relative liver weight attributable to the accumulation of hepatic hydroxyproline following resection in rats with bile obstruction-induced liver fibrosis (Layman *et al*., 2019). In a CCl4-induced liver damage model, relative liver weight was a more sensitive indicator of liver damage than the mean liver weight. In CC14 ingestion, fat from periphery adipose tissue is relocated to the liver, accumulating and increasing liver wet weight while the damp weight of adipose tissue diminishes (Neshat *et al*., 2021).

Furthermore, CCl_4 inhibits the production of apolipoproteins, leading to a reduction in lipoprotein synthesis. The current investigation indicated that three weeks of HE-DA therapy did not significantly differ from the untreated control regarding liver wet weights.(Li *et al*., 2024). CCl_4 combined with HE-DA treatment demonstrated a substantial ($p<0.05$) reduction in the liver's wet weights relative to those treated with CCl_4. The HE-DA treated groups exhibited a substantial ($p<0.05$) reduction in liver wet weights compared to the CCl_4 treated group, with no significant difference seen compared to the CCl_4 with Silymarin treated group. The injection of HE-DA markedly recovered body and liver weights, approaching those of the control group. Furthermore, HE-DA therapy demonstrated outcomes comparable to the reference medication.

CCl4 caused a significant increase in Total Cholesterol, Triglyceride Phospholipids, Free Fatty Acids, HDL, and LDL levels relative to control values after intoxication, as seen in Figure 2(A) and (b). The injection of HE-DA in CCl4-intoxicated rats decreased levels of Total Cholesterol, Triglycerides, Phospholipids, Free Fatty Acids, HDL, LDL, and VLDL. Likewise, administering HE-DA at 300 mg/kg to CCl4-intoxicated rats decreased Total Cholesterol, Triglycerides, Phospholipids, Free Fatty Acids, HDL, and LDL levels. Radical generation and lipid peroxidation are the principal cellular mechanisms behind CCl4-induced fatty liver growth. Substantial lipid accumulation is regarded as a harmful condition, and when it grows chronic, it results in fibrotic changes in the cells, progressing to cirrhosis and impaired liver function (Unsal *et al*., 2021).

The concentration of cholesterol, triglycerides, and free fatty acids was raised in plasma and tissues. CCl4 promotes the production of fatty acids and triglycerides from acetate. This may result from the translocation of acetate into the liver cell, resulting in increased substrate (acetate) availability. The synthesis of cholesterol additionally amplifies CCl_4 toxicity (Saleh *et al*., 2024).

The current investigation demonstrated that prolonged intermittent therapy with CCl_4 resulted in a substantial elevation ($p<0.05$) in plasma total cholesterol and triglyceride concentrations. Treatment of HE-DA at various dosages for three weeks did not exhibit significant differences ($p>0.05$) compared to the usual control for total cholesterol and triglycerides. However, CCl_4, in conjunction with HE-DA and Silymarin therapy, demonstrated a substantial ($p<0.05$) reduction in plasma levels of total cholesterol and triglycerides relative to the CCl_4-treated group. The administration of CCl_4 significantly elevated triglycerides, total cholesterol, LDL, and HDL values Figure 2(B). Elevated cholesterol levels may result from enhanced fatty acid esterification, suppression of fatty acid β-oxidation, and reduced excretion of cellular lipids. CCl_4 enhances acetate uptake into hepatic cells, likely by facilitating acetate accessibility and promoting cholesterol biosynthesis. (Chen *et al*., 2024)It also augments the production of fatty acids and triglycerides from acetate and promotes lipid esterification. Suppressing lysosomal lipase activity may result in triglyceride buildup in the liver (Carotti *et al*., 2020)Current research reveals elevated oxidative stress indicators in the liver of HE-DA hepatic cirrhotic rats, indicating increased oxidative stress in these organs. HE-DA therapy for 21 days has reduced the severity of oxidative stress indicators in rats with hepatic cirrhosis. Additionally, all seven experimental groups evaluate the antioxidant status and enzymes to elucidate HE-DA treatment's protective effect against CCl_4-induced oxidative stress in the liver.

Lipid peroxidation (LPO) in hepatic tissue was examined across seven experimental groups, with results shown in Figure 3. CCl_4-induced rats administered HE-DA (group V) and Silymarin (group VI) exhibited no significant elevation in liver lipid peroxidation (LPO). In contrast, group II (CCl_4 treated) demonstrated a significant increase ($p<0.05$) in Malondialdehyde (MDA) levels compared to the control group, while groups III, IV, V, and VI recorded significantly decreased levels ($p<0.05$).

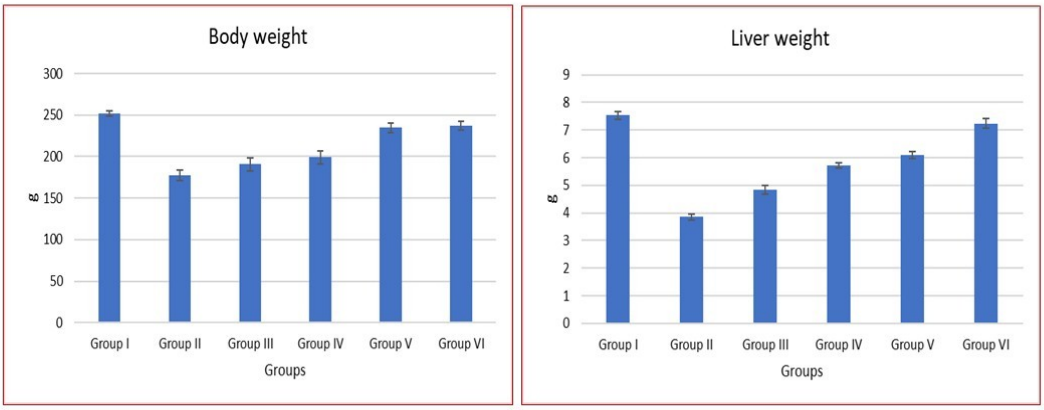
This study demonstrated that rats undergoing three weeks of intermittent CCl_4 treatment showed a significant increase in MDA levels in liver tissue relative to the normal control rats. Lipid peroxidation induced by CCl_4 mainly depends on the biological activation of the trichloromethyl radical and trichloromethyl peroxy radical (Unsal *et al*., 2021). The system of cytochrome P450 is acknowledged for activating carbon tetrachloride (CCl_4). The principal product is the trichloromethyl free radical, believed to initiate the metabolic pathways that ultimately result in liver cell necrosis (Xu *et al*., 2020). The trichloromethyl radical may make a covalent link with lipids and proteins, interact with O_2 to produce a trichloromethyl peroxy radical, or abstract hydrogen atoms to yield chloroform (Recknagel *et al*., 2020). Supplementary products include linked dienes, lipid hydroperoxides, malonaldehyde comparable, and other short-chain hydrocarbons. In response to hepatic injury induced by the biotransformation of CCl_4 into radical radicals, "activated" Kupffer cells in the liver secrete increased quantities of reactive oxygen species and other bioactive substances. Lipid peroxidation serves as a crucial marker of oxidative stress. The increase in liver MDA levels due to CCl_4 signifies elevated lipid peroxidation, leading to hepatic tissue damage and the insufficiency of antioxidant defence mechanisms to prevent the production of excess free radicals (Demirci-Cekic *et al*., 2022). Free radical scavenging is a fundamental antioxidative mechanism that inhibits the chain process of lipid peroxidation. The treatment with CCl_4 and HE-DA alleviated oxidative stress, as shown by reduced lipid hydroperoxide levels in the CCl_4 rat model. Lipid hydroperoxide concentrations were significantly decreased ($p<0.05$) compared to the CCl_4-treated group (Ullah *et al*., 2020). The results demonstrate that the elevated liver lipid peroxide levels induced by CCl_4 were rectified after treatment with HE-DA. Figure 4 depicts the concentrations of liver glutathione (GSH) among six experimental groups. Group II (CCl_4 treatment alone) demonstrated a significant ($p<0.05$) decrease in glutathione levels relative to healthy control rats (Group I). Nevertheless, Group V (CCl_4 administered with HE-DA at 300 mg/kg bw) and Group VI (CCl_4 administered with Silymarin) exhibited no significant difference ($p>0.05$) compared to the usual control. Groups V and VI had a statistically significant increase in glutathione levels ($p<0.05$) relative to Group II. Reduced glutathione (GSH) is an essential non-enzymatic antioxidant that detoxes several toxic substances. The reduction of GSH, due to several factors, ultimately promotes the generation of Reactive Oxygen Species (ROS) and oxidative stress, resulting in effects that undermine the functional and structural integrity of cellular and organelle membranes (He *et al*., 2017). Lipid peroxidation mediated by CCl_4 generates reactive oxygen species, including the superoxide anion O2-, hydrogen peroxide (H_2O_2), and hydroxyl radical (OH). Reactive oxygen species (ROS) compromise antioxidant defence mechanisms, lower intracellular concentrations of reduced glutathione, and lessen the capacity of superoxide dismutase (SOD) (Sharma *et al*., 2023). This study showed that rats undergoing three weeks of intermittent CCl_4 treatment had significantly ($p<0.05$) reduced hepatic glutathione levels (Figure 4). Over three weeks, treatments with HE-DA and Silymarin did not demonstrate significant changes ($p>0.05$) in liver glutathione levels compared to the untreated control. The combination of CCl_4 and HE-DA significantly ($p<0.05$) brought back liver glutathione levels compared to individuals treated with CCl_4. Subsequently, it was noted that hydroalcoholic extracts from several plants elevated cellular GSH levels and promoted de novo GSH synthesis in HSC by augmenting the function and gene expression of GCL (Glutamate-cysteine ligase), a vital rate-limiting enzyme in GSH formation (Smirne *et al*., 2022). The de novo synthesis of GSH was crucial for several plants' hydroalcoholic extractions to inhibit HSC activation. This study posited that hydroalcoholic extracts from multiple plants might reduce oxidative stress by protecting the liver from CCl_4-induced injury and fibrosis (Barakat & Almundarij, 2020). This study demonstrated that the oral administration of plant extracts increased the total hepatic GSH level and significantly improved the liver's GSH/GSSG ratio (Onyibe *et al*., 2021). Figure 4 presents an analysis of several antioxidant enzymes, including Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), and Glutathione-S-transferase (GST) activity in liver tissue across all groups. Group II (CCl_4 treated alone) had a significant ($p<0.05$) decrease in the activity of CAT, SOD, GPx, and GST relative to the Normal control group. In contrast, groups V and VI had a significant ($p<0.05$) rise in antioxidant activity relative to group II. Antioxidant activity, or the inhibition of free radical generation, is essential for protection against CCl_4-induced hepatic illness. The animal system has an effective defence mechanism to prevent and mitigate damage caused by free radicals. A suite of natural antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase, executes this role. These enzymes provide a synergistic defence strategy towards reactive oxygen species. Superoxide dismutase (SOD) is a metalloprotein that functions as the primary enzyme in the antioxidant defence system by diminishing the steady-state amount of superoxide anion (O_2) (Ervianti *et al*., 2024). GPx is a selenoenzyme, with two-thirds localised in the liver's cytosol and one-third in the mitochondria. It promotes the interaction between hydroperoxides and reduced glutathione, leading to the synthesis of glutathione disulphide (GSSG) and the reduction result of the hydroperoxide (Sharapov *et al*., 2021). Glutathione S-transferase (GST) is essential in the liver for detoxifying harmful chemicals and combining them with glutathione. CCl_4-induced hepatotoxicity disrupts the balance of reactive oxygen species (ROS) production and antioxidant defences, leading to oxidative stress that hinders cellular functions via a series of events, culminating in liver necrosis (Prysyazhnyuk *et al*., 2021). The toxicity of CCl_4 is often ascribed to the active free radical (CCl3), generated by its oxidative degradation by liver cytochrome P450 (Saha *et al*., 2024). The reactive intermediate is believed to facilitate lipid peroxidation and the deterioration of cellular membranes.

The present study revealed three weeks of HE-DA therapy did not show significant ($p>0.05$) variations in liver antioxidant enzyme levels compared to the untreated control. Treatment with CCl_4 led to an important ($p<0.05$) decrease in antioxidant enzymes, namely SOD, CAT, GPx, and GST levels in liver tissue (Figure 4). The amalgamation of CCl4 and HE-DA treatment showed a significant ($p<0.05$) enhancement in hepatic antioxidant enzyme levels compared to those administered CCl_4 only. The *in vivo* experimental studies conducted by the researchers above indicate that plant metabolites can alleviate oxidative stress in inflammatory conditions by downregulating nitric oxide production and scavenging free radicals, including superoxide anions and H_2O_2, which are involved in oxidative chain reactions. Furthermore, oxidative stress resulting from acute and sub-chronic inflammation diminishes the levels of assumed non-enzymatic (GSH) and enzymatic (GPx and SOD) antioxidants in the impacted tissues. The reduction of antioxidants seen in several experimental models was significantly restored by HE-DA treatment. Thus, the antioxidant activity of HE-DA may be directly or indirectly associated with preserving membrane integrity, therefore contributing to the avoidance of increased serum marker enzymes seen during inflammation.

The hepatocyte regions in Group II (CCl_4 control) and Groups III, IV, and V (HE-DA treated with 100, 200, and 300 mg/kg bw) had no changes relative to Group I (normal control). The liver lobules of the control groups (I, V, and VI) had a conventional architecture, with hepatocyte plates arranged from the portal triads to the central vein, where they interconnect freely. Hepatocytes are densely organised in cord-like structures devoid of vacuolisation. A centrally positioned nucleus characterises each hepatocyte. The central vein is transparent, with visible blood cells present. The nucleus is prominently pigmented and centrally positioned inside the hepatocytes. Conversely, liver slices from rats administered CCl_4 only (Group II) exhibited extensive alterations throughout the lobules, characterised by lipid accumulations, cellular vacuolisation, and centrilobular necrosis (Gandahi *et al*., 2023). The sinusoids were partially occluded. There is a collection of blood inside the central area, indicating a hemorrhagic disease of the liver. The aggregation of blood cells is seen. The nuclei were separated and disorganised. Group V (CCl_4 + HE-DA) demonstrated hepatocyte regeneration. Nonetheless, sinusoids are disjointed, and the nuclei exhibit necrotic conditions in some cells. Vacuolisation is also seen between the cords at extreme magnification. The nuclei are prominently situated centrally, devoid of vacuolisation among hepatocytes (Shyu & Ali, 2022). Group VI (CCl_4 + standard medication) had almost normal histology, characterised by well-defined hepatocytes and a regenerating central vein.

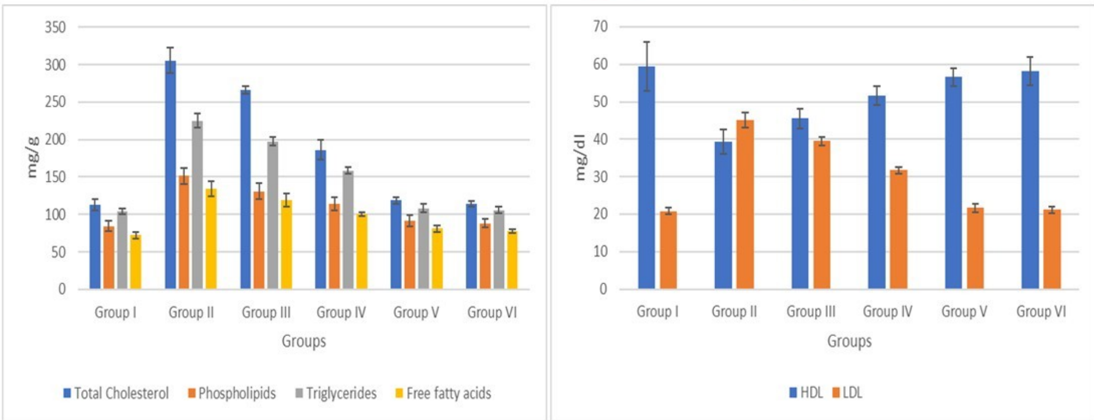
Histopathological tests were conducted to provide direct proof of the hepatotoxicity of CCl_4. The metabolism of CCl_4 in the liver stimulates lipid peroxidation and generates free radicals, leading to hepatocyte necrosis, inflammation, and the advancement of hepatic fibrogenesis. This research demonstrated that discontinuous treatment with CCl_4 (Group II, exposed to CCl_4 alone) for three weeks significantly altered hepatocyte structure in liver tissue. Hepatic tissue exhibits fatty accumulations, cellular vacuolisation, centrilobular necrosis, and congestion of the central vein. Light microscopic examination revealed hepatic hypertrophy, hepatocellular necrosis, and extensive fatty infiltration (Umarjon *et al*., 2023). The electron microscopic analysis revealed hepatocytes exhibiting dark heterochromatic (inactive) nuclei, with cytoplasmic fragmentation of rough endoplasmic reticulum, distributed glycogen granules, and many electron-lucent regions within the cytoplasm (Vani *et al*., 2024). These modifications may result from the harmful effects of CCl_4 metabolites, which have damaged many protein systems, including those in the rough endoplasmic reticulum and mitochondria.

Furthermore, reactive oxygen species induced the oxidation of cellular proteins and significant damage to mitochondrial DNA, compromising mitochondrial synthesis in liver cells (Venditti & Di Meo, 2020). Dao *et al*. (2021) also observed that blood sinusoids were clogged and loaded with red blood cells (Dao *et al*., 2021). The identified electron-lucent regions in the cytoplasm may represent lipid droplets. Lipids were abnormally deposited in the livers of rats with CCl_4-induced hepatotoxicity (Khan *et al*., 2023). The therapy of HE-DA in rats did not exhibit any differences compared to the normal control rats.



(A) **(B)**

Figure 1: The effect of HE-DA on (A) Body weight and (B) Liver weight of CCl_4- induced rats. The column bar signs indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group (P < 0.05).



(A) **(B)**

Figure 2: The effect of HE-DA on Lipid profile of CCl_4- induced rats. The column bar signs indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group (P < 0.05).

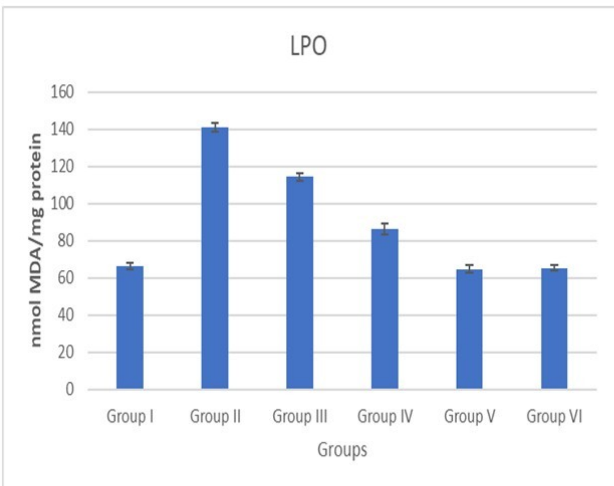


Figure 3: The effect of HE-DA on LPO of CCl_4- induced rats. The column bar signs indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group (P < 0.05).

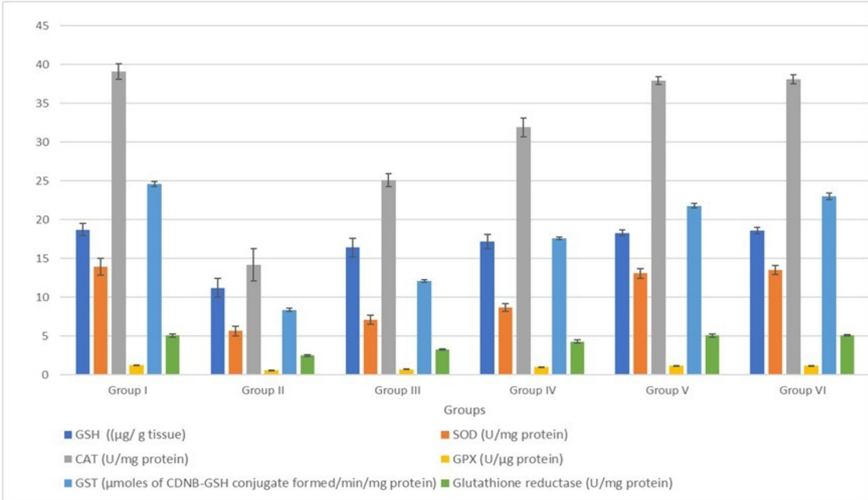


Figure 4: The effect of HE-DA on Antioxidant levels of CCl_4- induced rats. The column bar signs indicate mean ± standard deviation (n = 6). * shows the significance of differences relative to the normal control group ($P < 0.05$).

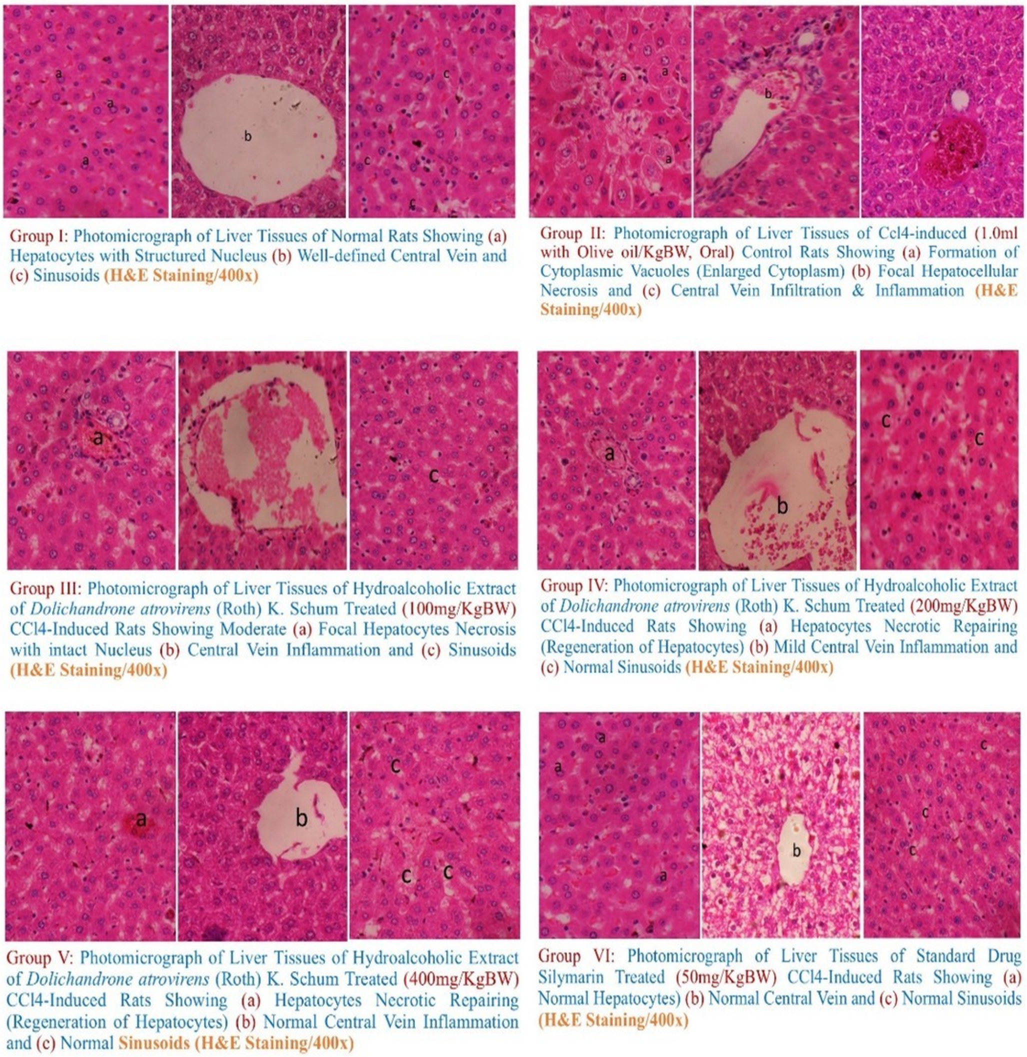


Figure 5: Photomicrograph of liver from (A) Group I showed normal histobasoligical structure of liver, (B) rats received $CC1_4$ (0.1 ml with olive oil/kg BW), (C) rats received $CC1_4$ (0.1 ml with olive oiUkg BW) and HE-DA (100 mg/kg BW), (D) rats received $CC1_4$ (0.1 ml with olive oiUkg BW) and HE-DA (200 mg/kg BW), (E) rats received $CC1_4$ (0.1 ml with olive oiUkg BW) and rats HE-DA (300 mg/kg BW) and (F) rats received CC14 (0.1 ml with olive oil Ukg BW) and Silymarin (50 mg/kg BW) (H&E x 400).

The treatment with CCl_4 in conjunction with HE-DA resulted in significant regeneration of hepatocytes, restoration of the central vein, and resolution of congestion in the liver tissue. Our results align with previous research. Shu-Ju Wu *et al*. (2008) found that oral treatment of HE-DA (100, 200, and 300 mg/kg) effectively restored the histological structure of the liver in cases of chronic CCl_4 intoxication and decreased hepatic hydroxyproline levels. Both findings indicated that HE-DA safeguarded the liver against fibrogenesis induced by CCl_4 in the rat model. HE-DA can reduce lipid peroxidation and enhance antioxidant enzyme activities. The liver's design restores normalcy via its function. Treatment with CCl_4 and HE-DA exhibited architecture almost indistinguishable from that of normal control rats. The reference treatment with CCl_4 and Silymarin exhibited an architecture almost indistinguishable from that of normal control rats. The current findings are promising, necessitating more investigations to validate the effectiveness of HE-DA and Silymarin.

CONCLUSIONS

This study's results indicate that hydroalcoholic extracts of *Dolichandrone atrovirens* leaves possess antioxidant properties and may safeguard the liver from damage caused by free radicals generated during CCl4 metabolism. The findings suggested that this plant extract may reduce elevated cholesterol and triglyceride levels, signifying its antihyperlipidemic characteristics.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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