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Original Article

Evaluation of *Mimosa pudica* Leaf Extract on Oxidative Stress and Fibrosis in Liver of Carbon Tetrachloride (CCl_4) Administered Rats

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Abstract: This study sought to investigate the protective effects of *Mimosa pudica* leaves extract on CCl_4-intoxicated hepatic inflammation and fibrosis in Long-Evans rats. Rat liver developed fibrosis, inflammation, and oxidative stress due to CCl_4 administration. Rats given CCl_4 showed increased levels of nitric oxide (NO), advanced oxidation protein product (AOPP), and malondialdehyde (MDA), and these were followed by decreased glutathione (GSH) levels and catalase activity. *Mimosa pudica* extract treatment showed significant reduction in oxidative stress parameters, lowered myeloperoxidase (MPO) activities, and improved endogenous antioxidant enzyme activities in rats intoxicated with CCl_4. Furthermore, *Mimosa pudica* as a treatment avoided the obvious expression of inflammation and fibrosis in the liver of CCl_4 administered rats. In conclusion, *Mimosa pudica* may prevent oxidative stress, inflammation, and fibrosis in the liver of rats given CCl_4; it is probable that increasing the activity of antioxidant enzymes is how this protective effect is achieved.

Keywords: *Mimosa pudica*, fibrosis; oxidative stress; carbon tetrachloride; malondialdehyde.

1. Introduction

Liver fibrosis is a medical condition which may develop in severe liver cirrhosis and may lead to hepatocellular carcinoma (HCC). A response to chronic liver injury brought on by several conditions, among these are cholestatic liver illnesses, alcohol consumption, non-alcoholic steatohepatitis (NASH), autoimmune hepatitis, viral hepatitis (hepatitis B and hepatitis C), and non-alcoholic fatty liver disease (NAFLD) [1]. The prevalence of nonalcoholic fatty liver disease (NAFLD) in Bangladesh is higher than that of its surrounding countries, affecting approximately one-third of the population and increasing their risk of liver-related illness and mortality [2,3]. The predominant attribute of liver fibrosis is the overabundance of type I collagen deposits, which arises from a complex transformation or activation process of hepatic stellate cells (HSCs), which change into an activated, myofibroblast-like cell that expresses α-smooth muscle actin (α-SMA) and secretes extracellular matrix made up of several proteoglycans and proteins after quitting as a cell that stores vitamin A [4,5]. By secreting transforming growth factor beta (TGF-β) and other agonists, macrophages and Kupffer cells in the liver, can stimulate fibrogenesis. However, they can also aid in the regression of fibrosis by secreting collagenases, which break down the fibrous scar [6]. When oxidative stress is present, Kupffer cells

typically release higher levels of cytokines, including transforming growth factor-1 (TGF-1), platelet-derived growth factor (PDGF), endothelin-1 (ET-1), and tumor necrosis factor-α (TNF-α). This process is a major contributor in the development of liver damage [7]. In addition, hepatic encephalopathy and other clinical problems such as hydropic decompensation and bleeding episodes are mostly brought on by persistent portal hypertension may also found in liver fibrosis [8]. Oxidative stress and inflammation are the hallmark indicators for the onset and advancement of hepatic dysfunction by viral infection and NAFLD [9]. When the natural antioxidant defense mechanism is unable to scavenge and remove reactive oxygen species (ROS), or free radicals, an abrupt increase in their levels results in oxidative stress and the emergence of illness [10]. Oxidative stress modifies immune cell responses, extracellular matrix composition, and parenchymal structure [11].

A common industrial solvent, carbon tetrachloride (CCl_4) is used to develop the most well-studied hepatotoxicity model caused by oxidative stress generated by xenobiotics in animals. The indications and manifestations of CCl_4-induced chronic liver injury are exactly like those of human chronic liver injury [12]. High concentrations of CCl_4 exposure can damage the kidney, liver, and central nervous system, resulting in coma or even death. Hepatotoxicity is caused by the reactive free radicals originates due to CCl_4 metabolism inside the liver, such as the trichloromethyl radical ($^{\bullet}CCl_3$). Rats with a single dosage of CCl_4 experience lipid alterations and cell necrosis, which are directly linked to the hepatic enzyme leaking into the blood [13]. The CYP450 enzyme system is primarily responsible for the conversion of CCl_4 into the free radicals such as peroxy trichloromethyl ($^{\bullet}OOCCl_3$) and trichloromethyl ($^{\bullet}CCl_3$), peroxy radicals like this encourage Kupffer cells to generate reactive oxygen species (ROS) like [$^{\bullet}O^{-}_2$], H_2O_2, and [•OH], resulting in lipid peroxidation and known to cause both acute and long-term tissue damage such as fibrosis, steatosis, necrosis, and hepatocarcinoma. In addition to Kuffer cells, CCl_4 administration draws additional inflammatory cells to the liver and exacerbates liver necrosis. Animals with CCl_4-mediated hepatic impairment also showed reduced antioxidant levels and a high concentration of lipid peroxidation products [14,15]. There is growing evidence that hepatic fibrosis is a condition that can be reversed. As a result, the hepatic fibrotic process would most likely be stopped or reversed by an efficient treatment [16].

Due to the affordability and dependability of medicinal plants, they are recognized as a safe and effective alternative medicine and a healthy source of life for all people [17]. Many researchers are now focusing on natural products to cure a variety of devastating conditions, such as liver malfunction, and they have shown encouraging protection in several *in-vivo* and *in-vitro* models [11]. *Mimosa pudica*, locally known as lojjaboti and referred to as a "sensitive plant" or "touch-me-not" in English, is one such therapeutic plant that has gained significance in South Asian countries [18]. It has historically been used to treat a variety of illnesses including neurasthenia, insomnia, tumor, traumatic injury, alopecia, pulmonary tuberculosis, dysentery, diarrhea, and various urogenital infections [19,20]. Mimosine, an alkaloid which has anticancer potential is found in *Mimosa pudica*, in addition with several prominent secondary metabolites, including triterpenes, steroids, flavonoids, tannins and glycosylflavones. The previous report suggested that a diverse range of pharmacological characteristics, including anti-inflammatory, anti-nociceptive, hepatoprotective, anti-bacterial, antiparasitic, anti-fungal, anticonvulsant, antidepressant, antimalarial, antidiarrheal, hypolipidemic, diuretic, and hypoglycemic effects, have been linked to various sections of *Mimosa pudica* [20,21,22]. *Mimosa pudica* showed strong antioxidant activities in difference *in vitro* assay systems [23]. A study suggested that *Mimosa pudica* chloroform extract demonstrated a notable hypolipidemic impact when administered at a dose of 200 mg/kg body weight on rats [24]. Considering these beneficial responses exerted by *Mimosa pudica* extract, this investigation was undertaken to assess its defensive beneficial effects against rat liver damage caused by CCl_4 administration.

2. Materials and Methods

2.1 Chemicals and reagents

Merck was the source of carbon tetrachloride (Darmstadt, Germany). The kits for alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) were made available by DCI Diagnostics

(Budapest, Hungary). The supplier of standards used in oxidative stress parameter assay, and additional reagents and Sirius red staining was Merck (Darmstadt, Germany). Thiobarbituric acid was obtained from the Sigma Aldrich (St. Louis, Missouri, United States of America). A supply of reduced glutathione (GSH) was obtained from J.T. Baker in New Jersey, USA. The SOD standard and further analytical components were procured from SR Group, located in Delhi, India. In all other cases, analytical grade chemicals and reagents were used.

2.2 Plant Sample Collection and Extraction

The *Mimosa pudica* plants utilized in this experiment were identified at the Bangladesh National Herbarium in Mirpur, Dhaka, from the village region in the Sherpur district of Bangladesh. A voucher specimen was preserved (DABC 99124).

After collection, the *Mimosa pudica* leaves were washed to remove any unwanted elements and debris, then allowed to dry naturally. Leaves were ground into a fine powder in a grinder machine after drying. To obtain the extract, this fine powder (200 g) was thereafter steeped in 95% ethanol for a week in a closed glass container. The concentrated extract was obtained by evaporating the ethanol in a rotary evaporator, following the extraction process involved maceration. Together with diets, this crude extract was employed as a therapy. The plant extract was stored for the duration of the experiment in a cool, dry, and dark location in an airtight container.

2.3 Experimental Animals

Twenty-four male Long Evans rats weighing between 200 and 220 grams were taken from North South University's animal home after obtaining the permission from IACUC committee. Each rat resided in a separate cage with a light and day cycle of 12 hours and a room temperature of 24 ± 2 °C. Every rat was provided with a regular pelleted food and free access to water. The Institutional Animal Care and Use Committee (IACUC) approved the experimental protocol; the approval number is 2022/OR-NSU/IACUC/0305.

- Group I (control): The control rats were fed standard pelleted food and received water for two weeks.
- Group II (CCl_4): Animals were given 1 mL/kg intragastrically twice a week for two weeks. The solution was CCl_4 (1:3 in olive oil).
- Group III (control + *Mimosa pudica*): Received 100 mg/kg *Mimosa pudica* extract every day for two weeks.
- Group IV (CCl_4 + *Mimosa pudica*): For two weeks, rats received 1 mL/kg dosage of CCl_4 (1:3 in olive oil) twice a week and subsequently received 100 mg/kg *Mimosa pudica* extract every day for two weeks.

Regular recording was done of body weight, water intake, and food intake. Following a 14-day period, ketamine hydrochloride (90 mg/kg intraperitoneally) was administered to all the rats in order to cause their sacrifice. The liver, kidney, and blood tissues had been taken right away. There were two reasons the organs were weighed and divided. For biochemical analysis, one half was kept at -18 °C, and the other half was kept in neutral buffer formalin (pH 7.4) for staining and histological examination. Sonification was used to homogenize the liver tissues, followed by centrifugation with phosphate-buffered solution and collection of the supernatants for biochemical tests. Before being stored in 1.5 mL microcentrifuge tubes at −18 °C for further biochemical testing, the drawn blood samples were centrifuged at 4000 rpm for 15 min at 4 °C in order to draw out the plasma.

2.4 Evaluation of liver function markers

Liver function enzymes were examined in the plasma that had been taken out of the blood, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities in compliance with the manufacturer's suggested protocol (DCI Diagnostics, Hungary).

2.5 Estimation of thiobarbituric acid reactive substances (TBARS)

Oxidative stress is indicated by the presence of thiobarbituric acid reactive substances (TBARS), a result of polyunsaturated fatty acid peroxidation. The technique outlined in previous studies was used to estimate the amounts of TBARS in liver and kidney tissue homogenates, and plasma [25]. The procedure involved heating, cooling, and measuring the absorbance of the clear supernatant at 535 nm against a reference blank. The reaction solution contained 0.1 mL of sample and 2 mL of TBA-TCA-HCL (thiobarbituric acid, trichloroacetic acid, and hydrochloric acid) reagent. Finally, the amount of TBARS in the samples was calculated using a standard (1, 1, 3, 3, Tetramethoxypropane) curve equation and expressed as nmol/mL or nmol/g.

2.6 Estimation of nitric oxide (NO)

The previously published methodology was followed to determine the level of nitric oxide, a marker for liver fibrosis caused by oxidative stress, in liver homogenates and plasma using modified Griess-illosvoy reagents [25,26].

2.7 Estimation of advanced protein oxidation products (APOP)

In order to measure the amounts of advanced oxidation protein products (AOPPs), which are thought to be a novel indicator of oxidative stress, previously reported techniques were followed [25,27]. To put it briefly, the reaction mixture's absorbance, which included potassium iodide, acetic acid, and plasma diluted in phosphate buffer solution, was measured immediately at 340 nm against a blank solution. Chloramine-T was used as standard solution which has linearity ranged from 0 to 100 nmol/mL at this wavelength. The values of AOPP were reported as nmol/mL chloramine-T equivalents.

2.8 Estimation of catalase (CAT) activity

As established by the previous technique, catalase (CAT) is a type of antioxidant enzyme that eliminates H_2O_2 produced under oxidative stress [25,26].

2.9 Estimation of myeloperoxidase (MPO) activity

Myeloperoxidase (MPO) is a sign of inflammation that was found by applying the previously mentioned procedure [25,26]. To summarize, the sample protein (10 µg) was added to a test tube having 0.53 mM o-dianisidine dihydrochloride and 0.15 mM H_2O_2 combination in PBS. Then, the absorbance changes of the reaction mixture at 460 nm was measured. MPO activity/mg protein was used to represent MPO activity.

2.10 Measurement of glutathione reduction (GSH)

According to a prior study, glutathione was measured [28]. After mixing the sample (1 mL) with approximately 1 mL of sulphosalicylic acid, the mixture was placed in an ice-filled container. The mixture underwent a 20-minute centrifugation at 8000 g and 4 °C. After centrifugation, 3 mL of sample, 0.2 mL of DTNB (100 mM), and 2.7 mL of phosphate buffer solution (0.1 M) were mixed together. To determine the GSH level, the absorbance at 412 nm was measured right away.

2.11 The Histopathology Process

For numerous days, a portion of the liver tissues were kept in formalin that has been buffered neutrally (NBF, 10% v/v). Before being covered in paraffin wax, these preserved tissues were exposed to a gradually xylene treatment. Glass slides were used to hold the 5-micron-thick slices of paraffin block tissues. Each section

underwent xylene deparaffinization, followed by a series of dehydration and rehydration processes using a series of graded alcohol. To identify the basic tissue architecture and the infiltration of inflammatory cells, the slices were lastly stained with hematoxylin and eosin. A light microscope was used to take all of the pictures at a magnification of 40× [29,30].

2.12 Statistical Analysis

Mean ± SEM was employed for each test parameter in the data computation. Graph Pad Prism 9 was used for all data analysis in this investigation. A One-Way ANOVA and a Tukey test were used to compare the different groups. All of the differences were taken into account at $p < 0.05$ for statistical significance.

3. Results

3.1: Effect on liver wet weight of the CCl_4 induced rats

The results of the investigation showed that there is no significant change in the liver wet weight of the CCl_4-intoxicated rats and control rats or *Mimosa pudica* treated rats. Rats intoxicated with CCl_4 were treated with *mimosa pudica*; as Fig. 1 illustrates, the liver's wet weight of the treated group did not significantly ($p > 0.05$) differ from that of the control group. Remarkably, rats given CCl4 did not significantly ($p>0.05$) increase in the wet weight of their liver (**Figure 1**).

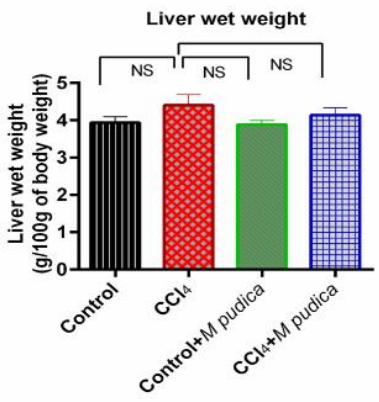

Figure 1. Impact of *Mimosa pudica* on rats given CCl_4 in terms of liver wet weight. Each value was displayed as mean ± standard error of mean (SEM) for a total of N = 6. During the statistical analysis, a one-way ANOVA and Tukey test were performed. A value of $p < 0.05$ is considered significant in all cases when it comes to statistical significance. In this instance, ns indicates $p > 0.05$.

3.2 Effect on liver marker enzymes AST, ALT, and ALP levels in plasma of the CCl_4 induced rats

In comparison to control rats, the degree of liver impairment caused by CCl_4 was significantly greater ($p < 0.01$). AST, ALP, and ALT plasma levels were increased in rats given CCl_4 (**Figure 2A, 2B, and 2C**). The CCl_4+ *Mimosa pudica* group demonstrated considerably lower plasma levels of ALT, AST, and ALP than the CCl_4 intoxicated group ($p < 0.01$), according to the study, which looked at the effect of *Mimosa pudica* extract on various biochemical markers (**Figure 2A, 2B, and 2C**). Additionally, compared to the control rats, the plasma ALT, AST, and ALP levels in the control+*Mimosa pudica* group were notably normal (**Figure 2A, B, and 2C**).

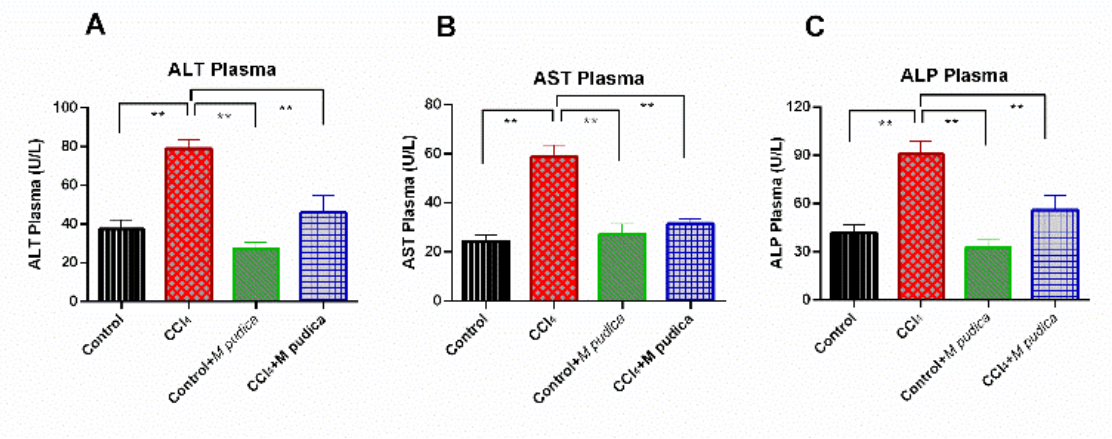

Figure 2: Effects of *Mimosa pudica* on rats given CCl_4 in terms of (A) ALT, (B) AST, and (C) ALP plasma levels. For each value, the mean ± standard error of mean (SEM) was employed. N is equivalent to 6. Statistical analysis included running a Tukey test and a one-way ANOVA. A value of $p < 0.05$ is considered significant in all cases when it comes to statistical significance. Here, $p > 0.05$ is denoted by ns, $p \leq 0.05$ by *, and $p \leq 0.001$ by **.

3.3 Mimosa pudica's impact on oxidative stress in rats administered CCl_4

The CCl4 group had considerably greater ($p < 0.01$) levels of MDA, a hallmark of oxidative stress, in their liver and plasma as compared to the control group, as depicted in **Figures 3A and 3D**. It is noteworthy to highlight those rats treated with *Mimosa pudica* exhibited noticeably reduced MDA levels **(Figure 3A and 3B**). MDA levels were similarly observed to be lower in the control+*Mimosa pudica* group compared to the CCl_4 group (**Figure 3A and 3B**).

A different oxidative stress signal, NO, was found at greater levels ($p < 0.01$) in the liver homogenates and plasma of the CCl_4 group when compared with the control group (**Figures 3B and 3E**). Comparing the CCl_4 group to the *Mimosa pudica*-treated rats, the CCl_4+ *Mimosa pudica* group demonstrated a substantial decrease ($p < 0.01$) in NO levels in the liver and plasma (**Figures 3B and 3E**). **Figure 3B and 3E** show that there was no significant decrease in the levels of NO in the plasma and liver between the control+ *Mimosa pudica* group and the control group.

Figures 3C and 3F indicate that the CCl_4 group's liver homogenates and plasma had significantly higher levels of AOPP than those of the control group did ($p < 0.01$). Importantly, rats receiving both *Mimosa pudica* and CCl_4 in contrast to the CCl_4 group, demonstrated significantly lower levels of AOPP in liver homogenates and plasma (**Figure 3C and 3F**). When comparing the control group to the control+*Mimosa pudica* group, there were no appreciable variations in the AOPP concentration in the liver homogenates or plasma (**Figures 3C and 3F**).

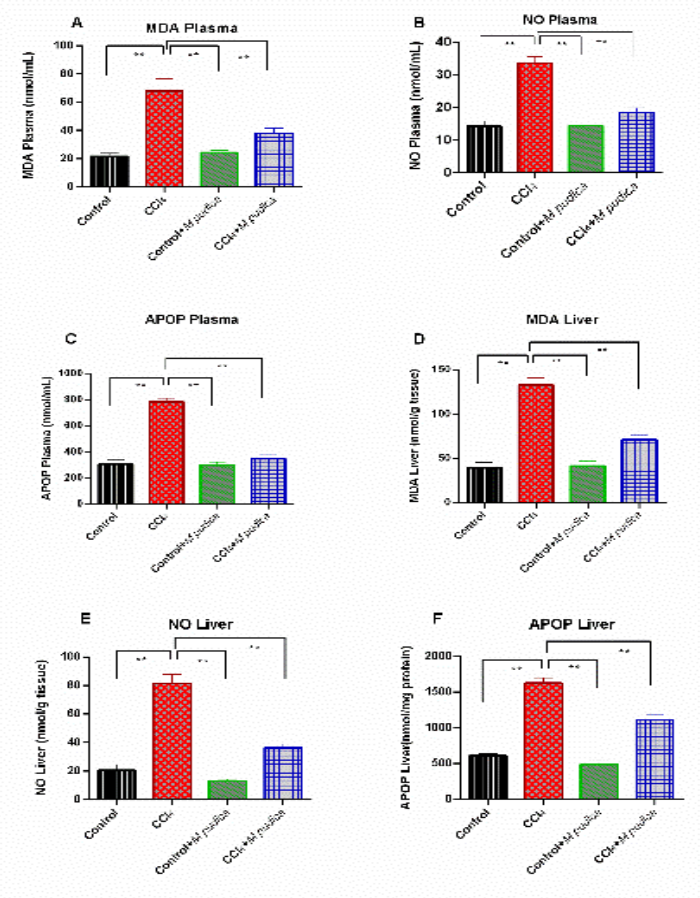

Figure 3: Effectiveness of Mimosa pudica on oxidative stress signs in rats administered CCl_4. This figure displays (A) MDA plasma, (B) NO plasma, (C) APOP plasma, (D) MDA liver, (E) NO liver, and (F) APOP liver. With N = 6, each value was shown as mean ± standard deviation (SD). Tukey test was performed after one-way ANOVA in statistical analysis. In terms of statistical significance, a value of $p \leq 0.05$ is deemed significant in every instance. Here, $p \leq 0.05$ by *, and $p \leq 0.001$ by **.

3.4 Impact of Mimosa pudica on the activity of antioxidant enzymes catalase in rats given CCl_4

When comparing the plasma and liver homogenates to the control group, the CCl_4 group had a significant ($p < 0.001$) decrease in catalase activity, an essential antioxidant enzyme (**Figure 4A and 4B**). Notably, animals receiving both CCl_4 and *Mimosa pudica* revealed significantly higher levels of catalase activity in liver homogenates and plasma when compared to the CCl_4 group ($p < 0.001$) (**Figure 4A and 4B**). The plasma and liver homogenates of the control+*Mimosa pudica* group weren't showing any appreciable differences in catalase activity when compared to the control group (**Figures 4A and 4B**).

3.5 Impact of *Mimosa pudica* on the activity of antioxidant enzymes GSH, and MPO activities in liver of rats given CCl_4

Following therapy with *Mimosa pudica*, GSH, another tissue antioxidant, was recovered in plasma and tissues. Unlike the rats in the group under control, the GSH level in plasma and liver of CCl_4 group was shown to be considerably lower ($p \leq 0.05$) (**Figure 5A and 5B**). After receiving treatment with *Mimosa pudica*, rats in the CCl_4-intoxicated group showed a significant ($p < 0.01$) recovery in their lowered plasma GSH level (**Figure 5A and 5B**). The control+ *Mimosa pudica* group showed no changes in plasma and hepatic GSH levels in comparison with the control group (**Figure 5A and 5B**).

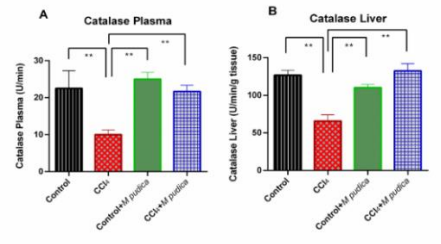

Figure 4: Impact of *Mimosa pudica* on the activity of antioxidant enzymes catalase in rats given CCl_4. (A) Catalase plasma and (B) Catalase liver are shown. Each value was represented by the mean ± standard error of mean (SEM). The statistical analysis procedure included running a one-way ANOVA and a Tukey test. N is equal to 6. When it comes to statistical significance, every result of $p \leq 0.05$ is considered significant. Here, $p \leq 0.05$ by *, and $p \leq 0.001$ by **.

The study revealed that the liver MPO levels of the CCl_4-intoxicated group was significantly ($p < 0.01$) higher in comparison to that of the control group (**Figure 5C**). The treatment of *Mimosa pudica* to the CCl_4 group (CCl_4+ *Mimosa pudica*) declined the MPO activity in liver significantly ($p \leq 0.01$) (**Figure 5C**). Furthermore, the liver MPO of the control+ *Mimosa pudica* group was normal in comparison to that of the control rats (**Figure 5C**).

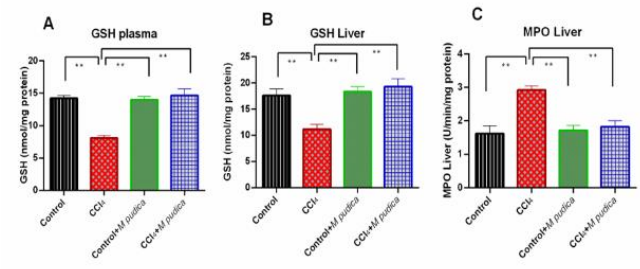

Figure 5: Impact of *Mimosa pudica* on (5A) GSH Plasma, (5B) GSH Liver, and (5C) MPO liver in rats given CCl_4. For each value, the mean ± standard error of mean (SEM) was utilized. N is equal to 6. The statistical analysis was carried out employing one-way ANOVA and a Tukey test. Any scenario where $p \leq 0.05$ is considered as statistical significance. Here, $p \leq 0.05$ by *, and $p \leq 0.001$ by **.

3.6 Impact of Mimosa pudica on the histopathology of the liver in rats given CCl_4

The findings related to liver histology in the *Mimosa pudica* -treated rats are illustrated in **Figure 6**. The findings suggested that there was no evidence of hepatic inflammation in the livers taken out of the rats in the control group (**Figure 6A**). On the other hand, the group that received CCl_4 treatment showed significant accumulation of collagen and hepatic inflammation (**Figure 6B**). Regarding the lobule and hepatocytes, the control+ *Mimosa pudica* showed typical structure and orientation (Figure 8B). *Mimosa pudica* treatment had the ability to decrease collagen deposition, repair hepatic necrosis and inflammation, and enhance liver function in rats (**Figure 6D**).

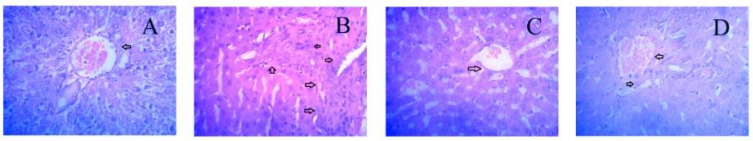

Figure 6: Effectiveness of *Mimosa pudica* on the histology of liver of rats after CCl_4 administration. Hematoxylin and eosin staining are shown in this picture from A to D. Here, A - Control; B - CCl_4; C - Control+ *Mimosa pudica*; and D - CCl_4+ *Mimosa pudica*. At a magnification of 40×, every image was photographed.

4. Discussion

Industrial toxin CCl_4 can cause morphological and functional changes that could eventually result in hepatotoxicity through processes such as production of free radicals, metabolic activation of compounds, lipid peroxidation, disturbance of calcium homeostasis, and covalent binding [15]. Due to the induction of CCl_4, there was an increase in various hepatic parameters such as AST, ALP, and ALT. Liver impairment is mostly determined by the elevated transaminase activities. The administration of CCl_4 led to the inhibition of phosphatases and transaminases, resulting in liver cell necrosis [31]. The analysis of the research presented that the administration of CCl_4 significantly increased the activities of blood marker enzymes like ALT, AST, and ALP. This finding is in line with other findings showed that rats with severe acute liver injury due to CCl_4 administration possess elevated AST and ALT activity [32]. This investigation also showed that treatment with *Mimosa pudica* extract may prevent hepatic damage and normalized transaminases activities in CCl_4 administered rats.

The primary source of tissue damage in the hepatic dysfunction model in animals administered with CCl_4 is oxidative stress. Liver cytochrome P450 breaks down CCl_4 to produce the free radical $\bullet CCl_3$ [33]. Free radicals' generation has the potential to damage cellular organelles and cause cell membrane rupture due to lipid oxidation. Free radical-induced hepatic cell damage can lead to an increase in the production of lipid peroxidation products, primarily malondialdehyde (MDA), in the liver and can also trigger an oxidative stress response [15,25]. The oxidative stress-related markers in the liver of rats received CCl_4 was increased, which may have been caused by a decrease in the activity of antioxidant enzymes (catalase and GSH) in the liver and plasma [26]. According to our findings, *Mimosa pudica* extract may be able to prevent hepatic damage brought on by CCl_4. Other researchers also found similar outcomes, showing hepatocellular regeneration and producing great defense against liver injury at various doses (300, 500, and 600 mg/kg) by lowering increased levels of hepatic parameters [34,35,36]. As one of the key markers of oxidative stress is lipid peroxidation, it damages hepatocellular components irreversibly. Additionally, MDA increases protein oxidation and weakens the lysosomal membrane, both of which harm organs [37]. Additionally, the results demonstrated that the *Mimosa pudica* extract reduced MDA levels in comparison to the CCl_4 group, which strongly suggests that *Mimosa pudica* has anti-lipid peroxidation properties. Antioxidant and anti-lipid peroxidation qualities of *Mimosa pudica* extract may be due to the presence of flavonoids and other polyphenolic compounds that make up its composition [38,39,40].

On the other hand, being a signaling molecule in biological systems, nitric oxide is an essential physiological component. However, nitric oxide instantly interacts with superoxide to form highly reactive peroxynitrite ($^{-}ONOO^{\bullet}$) when combined with additional ROS (superoxide anion [$^{-}O^{\bullet}{}_2$]), which may lead to nitrosative stress in tissues [41]. Despite its important function as a signaling molecule and in maintaining vascular tone, a further oxidative stress agent is nitric oxide. Nevertheless, scavenging nitric oxide can reverse hepatic damage caused by accelerated generation of nitric oxide by iNOS, or inducible nitric oxide synthase [42]. Moreover, the nitrosative stress induced by reactive nitrogen species (RNS) may also induce tissue toxicity and inflammatory conditions by damaging and disrupting cells [43]. Rats given CCl_4 showed considerably higher levels of nitric oxide in their liver tissue and plasma when compared to the control group. The increased nitrate concentration in the plasma and tissues of rats given CCl_4 is also decreased by *Mimusa pudica* treatment. A different stress indicator is called advanced oxidation protein product (AOPP). Increased levels of AOPP have been linked to hepatic dysfunction in patients with acute liver failure and non-alcoholic steatohepatitis (NASH) [44,45]. Rats under control were compared to which were also treated with *Mimosa pudica* to lower their AOPP concentration, the CCl_4-administered animals showed a substantial rise in APOP concentration in both their liver and plasma tissues.

A CCl_4 overdose alters the activity of antioxidant enzymes [46]. One significant antioxidant enzyme that scavenges free radicals produced in tissue is called catalase. All aerobic cells include the hemeprotein catalase enzyme, which breaks down H_2O_2 into oxygen and water. According to our study, CCl_4 administration reduces catalase activity, which is then restored to almost normal levels by *Mimosa pudica* extract treatment. An essential

part of cellular defense against reactive free radicals and other oxidant species is the GSH antioxidant system. Through its covalent attachment to •CCl_3 radicals and enhancement of the activities of glutathione reductase and glutathione peroxidase, GSH plays a crucial role in defending cells in opposition to damages caused by CCl_4 [47,48]. In this work, *Mimosa pudica* extract treatments returned the decreased GSH levels back to their normal levels, implying that *Mimosa pudica* extract may prevent the oxidative stress induced by CCl_4.

Furthermore, the MPO activity indicated the level of inflammation and functioned as a marker for the neutrophilic infiltration. The level of MPO enzyme and oxidative stress are closely related [49]. When the MPO generates oxidants, they react with a wide range of biological components, such as, amino groups, lipids, and DNA and may cause tissue damage at the inflammatory sites [29]. The current results indicate that rats given CCl_4 showed more MPO activity than the control rats, whereas, *Mimosa pudica* extract reduced the high MPO activity.

Additionally, according to this investigation, CCl_4 administration results in an influx of inflammatory cells into the rat liver. Hepatic stellate cells are generally activated by a variety of pro-inflammatory and inflammatory mediators in the hepatic tissues, which are produced by Kupffer cells, which are local macrophage type cells in the liver [50]. A histological analysis further revealed that, in comparison to normal rats, CCl_4-administered rats showed a high infiltration of inflammatory cells. In contrast, *Mimosa pudica* extract treatment significantly decreased the immigration of inflammatory cells as compared CCl_4- administered rats. Approximately 0.6 g/day was the dose employed in this investigation, based on comparisons of the body surface areas of humans and rats. The daily consumption of polyphenols is approximately 1 gram [51]. The amount of *Mimosa pudica* utilized in this study is therefore appropriate for usage in people.

5. Conclusions

Based on the discussion, it may be inferred that extracts from *Mimosa pudica* may provide protection against CCl_4-induced liver injury. Increased capacity for antioxidant defense, a reduced response to inflammation, and oxidative stress in the liver tissues may all contribute to this therapeutic impact. These results demonstrate the potential of *Mimosa pudica* as a useful component to stop liver damage caused by ROS. To fully understand the molecular mechanism of *Mimosa pudica*, more research is required.

Author Contributions: The concept and design of this study was generated by MNI, NS and MAA. MAA, MNI and NS also trained MA, IBM, IA, ABJ, TA, AA, SAK and MSA on all the research related activities and supervised and coordinated the whole study. MA, IBM, IA, ABJ and SAK carried out animal handling, animal experimentation and animal sacrifice. IBM, IA, TA, ABJ and SAK also performed the biochemical analysis. SAK, MA, IA, ABJ performed the histological analyses. Statistical analysis and result interpretation were done by MSA, MNI, NS, and MAA. The draft manuscript was prepared by MA, ABJ, TA, NS, MAA, MSA, and MNI. After reading the published version of the manuscript, all writers have given their approval.

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