EVALUATION OF THE HEPATOPROTECTIVE POTENTIAL OF ALLANTOIN AGAINST CARBON TETRACHLORIDE (CCL_4)-INDUCED LIVER DAMAGE IN WISTAR RATS

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Abstract

Liver injury caused by xenobiotics remains a major clinical concern due to the liver's central role in metabolism and detoxification. Carbon tetrachloride (CCl_4) is a well-established hepatotoxin that induces liver damage primarily through oxidative stress and lipid peroxidation. The present study was designed to evaluate the hepatoprotective potential of allantoin against CCl_4-induced liver injury in Wistar rats.

The intraperitoneal injection of CCl_4 caused hepatotoxicity. The animals were split into five groups: two groups treated with low and high dosages of allantoin, a standard group treated with silymarin, a CCl_4 control group, and a normal control group. Serum biochemical indicators such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein were measured in order to evaluate hepatic damage. In liver tissue, oxidative stress markers such reduced glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) were assessed. To evaluate structural changes in hepatic architecture, histopathological analysis was carried out.

Administration of CCl_4 resulted in a significant elevation of serum liver enzymes and lipid peroxidation, along with depletion of endogenous antioxidant defenses and marked histopathological damage. Treatment with allantoin significantly restored altered biochemical parameters, reduced oxidative stress, and improved antioxidant enzyme levels in a dose-dependent manner. Histological findings further confirmed the protective effect of allantoin, showing reduced hepatocellular necrosis, fatty degeneration, and inflammatory infiltration. The hepatoprotective effects of allantoin were comparable to those of the standard drug silymarin.

The results of this study suggest that allantoin possesses significant hepatoprotective activity against CCl_4-induced liver injury, primarily mediated through its antioxidant and membrane-stabilizing properties. Allantoin may serve as a promising natural therapeutic agent for the management of liver disorders.

Keywords: *Allantoin; Hepatoprotective activity; Carbon tetrachloride; Oxidative stress; Antioxidant enzymes; Wistar rats; Liver injury*

1. INTRODUCTION

The liver plays a pivotal role in maintaining metabolic homeostasis through its involvement in carbohydrate, lipid, and protein metabolism, detoxification of xenobiotics, and synthesis of plasma proteins. Owing to these functions, the liver is highly vulnerable to injury caused by drugs, environmental pollutants, alcohol, and industrial chemicals. Hepatic disorders remain a major global health concern, contributing significantly to morbidity and mortality worldwide **(Parasuraman et al., 2010)**.

Drug- and toxin-induced liver injury is one of the most common causes of acute and chronic liver disease. Among experimental hepatotoxins, carbon tetrachloride (CCl_4) is widely employed to induce liver injury in laboratory animals due to its reproducible pathological features that closely resemble human hepatic damage **(Diehl et al., 2001).** CCl_4 is metabolized in the liver by cytochrome P450 enzymes, particularly CYP2E1, into highly reactive trichloromethyl (CCl_3) and trichloromethyl peroxy ($OOCCl_3$) radicals. These free radicals initiate lipid peroxidation of polyunsaturated fatty acids in hepatocyte membranes, leading to membrane disruption, enzyme leakage, mitochondrial dysfunction, and hepatocellular necrosis **(Reitman & Frankel, 1957, Burtis & Ashwood, 1999)**.

Oxidative stress plays a central role in the pathogenesis of CCl_4-induced hepatotoxicity. Excessive generation of reactive oxygen species (ROS) overwhelms endogenous antioxidant defense mechanisms such as superoxide dismutase (SOD), catalase, and reduced glutathione (GSH), resulting in enhanced lipid peroxidation and cellular injury **(Pratt & Kaplan, 2000)**. Therefore, agents possessing antioxidant and free radical–scavenging properties are considered promising candidates for hepatoprotection.

Allantoin is a naturally occurring nitrogenous compound (diureide of glyoxylic acid) found in several medicinal plants, including *Symphytum officinale*, and is also produced endogenously through purine metabolism. Pharmacologically, allantoin is known for its wound-healing, anti-inflammatory, moisturizing, and antioxidant properties **(Friedman, 2003).** Previous studies have reported that allantoin enhances tissue regeneration, reduces oxidative stress, and modulates inflammatory responses.

Despite these beneficial properties, limited scientific evidence is available regarding its hepatoprotective efficacy in toxin-induced liver injury models.
Given the pivotal role of oxidative stress in liver damage and the antioxidant potential of allantoin, the present study was designed to evaluate the hepatoprotective effect of allantoin against CCl_4-induced liver injury in Wistar rats using biochemical markers, antioxidant parameters, and histopathological assessment **(Recknagel et al., 1989)**.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Adult health Both sexes of Wistar albino rats weighing 200–250 g were purchased from a facility that was permitted to maintain animals. The animals were kept in conventional laboratory settings (temperature 22 ± 2 °C, relative humidity 55 ± 5%, and a 12-hour light/dark cycle) for a week before the experiment. Water and a standard pellet diet were given freely. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, recommendations were followed for all experimental methods. **(CPCSEA, 2014)**.

2.2 Chemicals and Drugs

Allantoin was obtained in analytical grade purity. Carbon tetrachloride (CCl_4) and olive oil were purchased from standard chemical suppliers. Silymarin was used as a reference hepatoprotective drug. All other reagents and chemicals used were of analytical grade.

2.3 Induction of Hepatotoxicity

Hepatotoxicity was induced by intraperitoneal administration of carbon tetrachloride (1 mL/kg), diluted in olive oil in a 1:1 ratio. This method is widely accepted for inducing acute liver injury characterized by oxidative stress, inflammation, and hepatocellular necrosis **(Diehl et al., 2001, Halliwell & Gutteridge, 2015)**.

2.4 Experimental Design

Animals were randomly divided into five groups, each containing six rats:

1. **Group I (Normal Control):** Received vehicle only
2. **Group II (CCl_4 Control):** Received CCl_4 to induce hepatotoxicity
3. **Group III (Standard):** Received CCl_4 + silymarin (100 mg/kg, p.o.)
4. **Group IV (Test I):** Received CCl_4 + allantoin (50 mg/kg, p.o.)
5. **Group V (Test II):** Received CCl_4 + allantoin (100 mg/kg, p.o.)

All treatments were administered orally once daily for 14 days. The dosing regimen was selected based on earlier pharmacological studies demonstrating hepatoprotective and antioxidant activity **(Araújo et al., 2010)**.

2.5 Collection of Blood and Serum Biochemical Analysis

At the end of the experimental period, animals were fasted overnight and anesthetized using light ether anesthesia. Blood samples were collected by cardiac puncture, a widely accepted terminal blood collection method in laboratory rodents that allows the collection of sufficient blood volume for multiple biochemical estimations while minimizing hemolysis when performed under anesthesia **(Lee et al., 2018)**.

After being collected, the blood was placed in dry, clean centrifuge tubes and left to coagulate at room temperature. Centrifugation at 3000 rpm for 10 minutes was used to separate the serum, which was then kept at 4 °C until additional analysis. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein were among the serum biochemical parameters that were measured using commercially available diagnostic kits in accordance with the manufacturer's instructions and common clinical chemistry techniques. **(Kim et al., 2016).**

Elevated levels of serum transaminases and alkaline phosphatase were considered indicators of hepatocellular injury and compromised membrane integrity, whereas alterations in bilirubin and total protein levels reflected impaired hepatic excretory and synthetic functions, respectively **(Brent & Rumack, 1999)**.

2.6 Assessment of Oxidative Stress Parameters

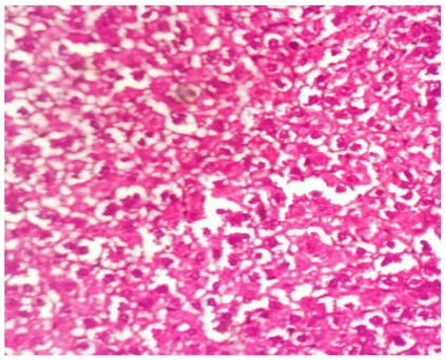
After removing the liver tissues, they were homogenized in phosphate buffer (pH 7.4) and cleaned with ice-cold saline. Oxidative stress indicators were estimated using the homogenate. Malondialdehyde (MDA) levels were estimated using the thiobarbituric acid reactive substances (TBARS) method in order to quantify lipid peroxidation. The ability of superoxide dismutase (SOD) to prevent the autoxidation of adrenaline was used to measure SOD activity. **(Reitman & Frankel, 1957)**, while reduced glutathione (GSH) levels were estimated using the Ellman method **(Pratt & Kaplan, 2000)**.

2.7 Histopathological Examination

Hematoxylin and eosin (H&E) was used to stain the liver tissues after they were fixed in 10% neutral buffered formalin, processed using normal histological techniques, embedded in paraffin, and sectioned at a thickness of 5 µm. Under a light microscope, sections were inspected for pathological alterations like necrosis, fatty degeneration, inflammation, and architectural deformation. **(Ohkawa et al., 1979)**.

2.8 Statistical Analysis

The mean ± standard deviation (SD) was used to express all data. One-way analysis of variance (ANOVA) and the relevant post-hoc tests were used for statistical analysis. Statistical significance was defined as a p-value of less than 0.05. **(Misra & Fridovich, 1972)**.



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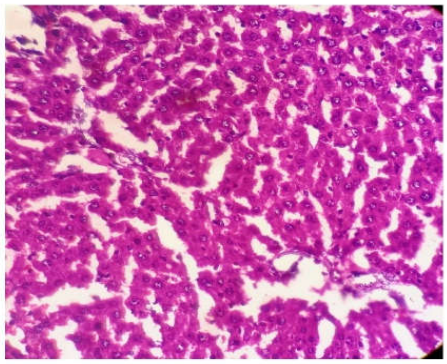

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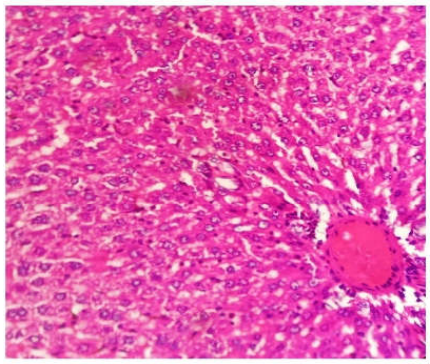

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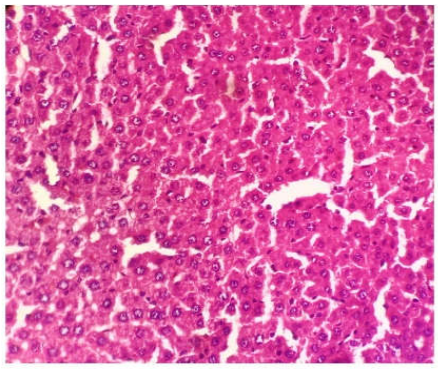

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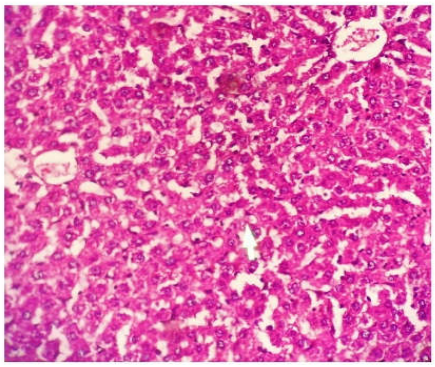

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Fig 1: - Effect of allantoin on liver histology

RESULT

3. Biomarkers of Liver Function

3.1 Effect of Allantoin on ALP Level in CCl4-Induced Hepatotoxicity in Rats

Table 1. Effect of treatments on ALP levels (U/L) in rat liver (Mean ± SD)

S.No.	Treatment Group	ALP Level (U/L)
1	Vehicle Control	50.61 ± 0.15
2	CCl4	201.58 ± 0.211
3	Allantoin Low Dose + CCl4	142.3 ± 0.44
4	Allantoin High Dose + CCl4	111.56 ± 0.17
5	Standard (Silymarin) + CCl4	95.55 ± 0.017

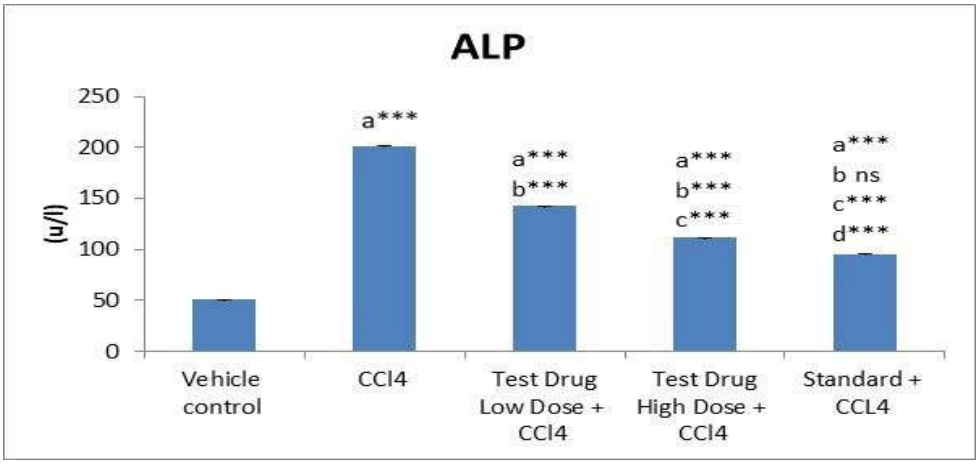

Figure 2. Graphical representation of ALP levels

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed significant elevation in ALP in the CCl4 group ($P < 0.001$) vs. vehicle control. Treatment with allantoin (low and high dose) and standard drug showed a significant reduction ($P < 0.001$) compared to the CCl4 group. The high-dose allantoin group was highly significant compared to both the CCl4 and standard groups ($P < 0.001$).

3.2 Effect of Allantoin on AST (SGOT) Levels in CCl4-Induced Hepatotoxicity in Rats

Table 2. Effect of treatments on AST levels (U/L) in rat liver (Mean ± SD)

S.No.	Treatment Group	AST Level (U/L)
1	Vehicle Control	50.58 ± 0.121
2	CCl4	119.85 ± 0.025
3	Allantoin Low Dose + CCl4	99.33 ± 0.205
4	Allantoin High Dose + CCl4	80.36 ± 0.013
5	Standard (Silymarin) + CCl4	58.45 ± 0.243

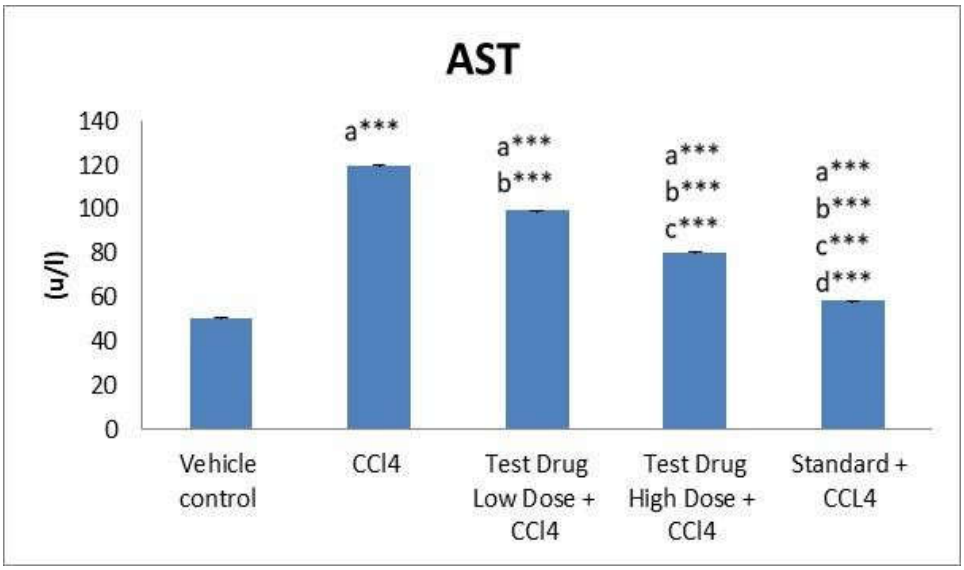

Figure 3. Graphical representation of AST levels

Results showed significant elevation in AST levels in the CCl4 group ($P < 0.001$) compared to vehicle control. Treatment with allantoin and standard drug significantly reduced AST levels ($P < 0.001$). High-dose allantoin demonstrated a stronger protective effect ($P < 0.001$) vs. both disease and standard groups.

3.3 Effect of Allantoin on ALT (SGPT) Levels in CCl4-Induced Hepatotoxicity in Rats

Table 3. Effect of treatments on ALT levels (U/L) in rat liver (Mean ± SD)

S.No.	Treatment Group	ALT Level (U/L)
1	Vehicle Control	41.82 ± 0.017
2	CCl4	74.3 ± 0.09
3	Allantoin Low Dose + CCl4	65.3 ± 0.15
4	Allantoin High Dose + CCl4	56.74 ± 0.013
5	Standard (Silymarin) + CCl4	45.24 ± 0.09

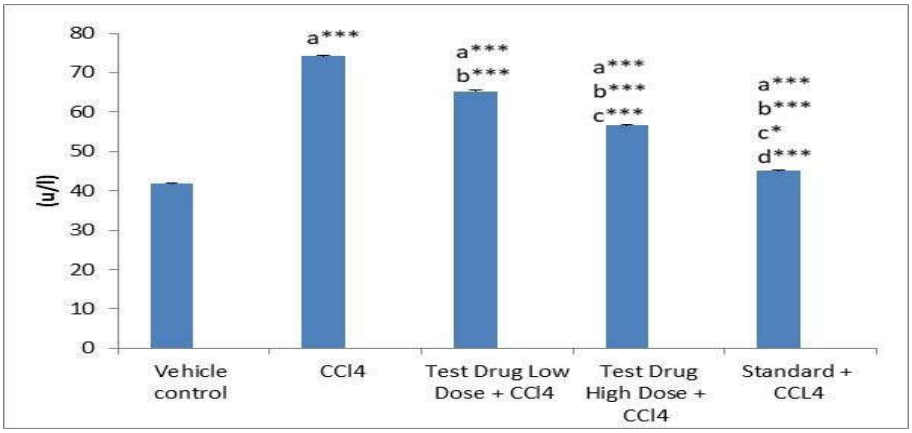

Figure 4. Graphical representation of ALT levels

ALT levels were significantly increased in the CCl4 group ($P < 0.001$). Both allantoin-treated and standard-treated groups showed a statistically significant reduction ($P < 0.001$). High-dose allantoin was highly effective ($P < 0.001$) compared to the toxic group.

3.4 Effect of Allantoin on Albumin Levels in CCl4-Induced Hepatotoxicity in Rats

Table 4. Effect of treatments on albumin levels (g/dL) (Mean ± SD)

S.No.	Treatment Group	Albumin Level (g/dL)
1	Vehicle Control	5.45 ± 0.170
2	CCl4	9.666 ± 0.179
3	Allantoin Low Dose + CCl4	7.35 ± 0.170
4	Allantoin High Dose + CCl4	6.616 ± 0.211
5	Standard (Silymarin) + CCl4	4.5 ± 0.216

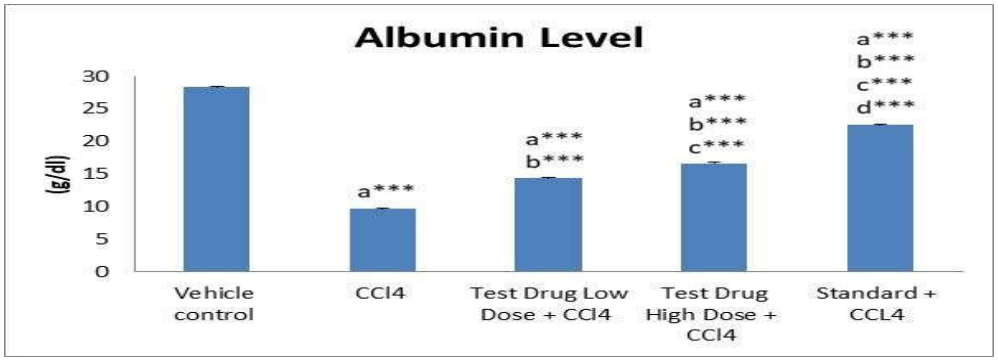

Figure 5. Graphical representation of albumin levels

Albumin was significantly elevated in the CCl4 group. Treatment groups (low and high dose) showed significant improvement, with the high dose group being highly significant ($P < 0.001$). The standard drug group also showed correction.

3.5 ANTIOXIDANT DEFENSE AGAINST OXIDATIVE STRESS

3.5.1 Effect of Allantoin on GSH Levels in CCl4-Induced Hepatotoxicity in Rats

Table 5. Effect of treatments on GSH levels (nmol/mg protein) (Mean ± SD)

S.No.	Treatment Group	GSH Level
1	Vehicle Control	20.73 ± 0.14
2	CCl4	11.94 ± 0.025
3	Allantoin Low Dose + CCl4	12.89 ± 0.02
4	Allantoin High Dose + CCl4	15.65 ± 0.023
5	Standard (Silymarin) + CCl4	17.89 ± 0.29

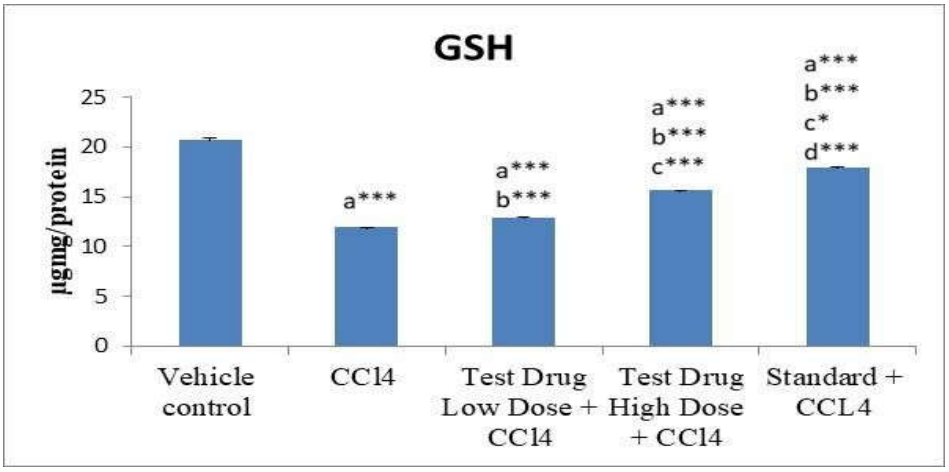

Figure 6. Graphical representation of GSH levels

GSH levels were significantly depleted in the CCl4 group ($P < 0.001$). All treatment groups showed statistically significant recovery ($P < 0.001$), with the high-dose allantoin group being highly significant vs. both the disease and standard groups

3.6 Oxidative Stress Markers

3.6.1 Effect of Allantoin on MDA Levels in CCl4-Induced Hepatotoxicity in Rats

Table 6. Effect of treatments on MDA levels (nmol/mg protein) (Mean ± SD)

S.No.	Treatment Group	MDA Level
1	Vehicle Control	3.333 ± 0.179
2	CCl4	5.35 ± 0.170
3	Allantoin Low Dose + CCl4	2.5 ± 0.129
4	Allantoin High Dose + CCl4	1.35 ± 0.170

5	Standard (Silymarin) + CCl4	2.416 ± 0.134

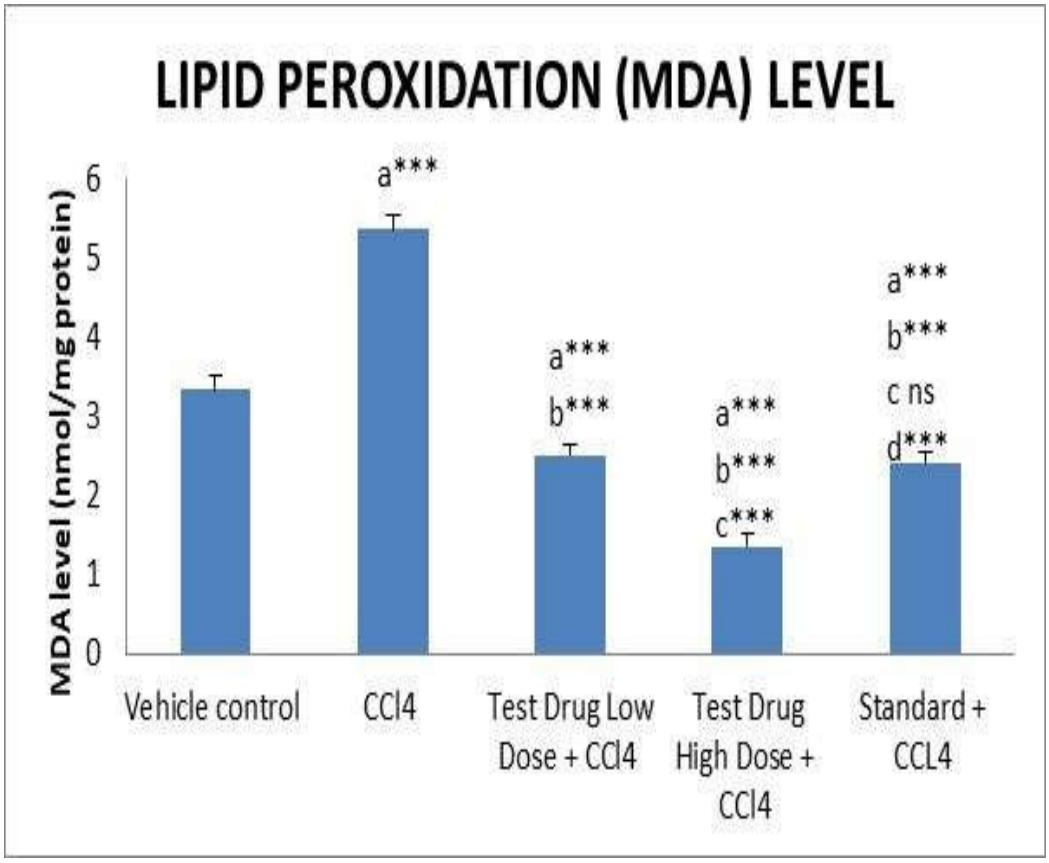

Figure 7. Graphical representation of MDA levels

Significant increase in MDA was observed in the CCl4 group vs. vehicle control ($P < 0.001$). Allantoin treatment (low and high dose) and standard significantly reduced MDA ($P < 0.001$), with the high dose being most effective.

3.6.2. Effect of Allantoin on SOD Levels in CCl4-Induced Hepatotoxicity in Rats

Table 7. Effect of treatments on SOD levels (U/mg protein) (Mean ± SD)

S.NO.	**Treatment group**	**SOD level in liver**
1	Vehicle Control	75.333 ± 3.204
2	CCl4	24.833 ± 4.44

3	Test Drug Low Dose + CCl4	42 ± 3.57
4	Test Drug High Dose + CCl4)	54.166 ± 3.188
5	Standard + CCl4)	45.333 ± 3.011

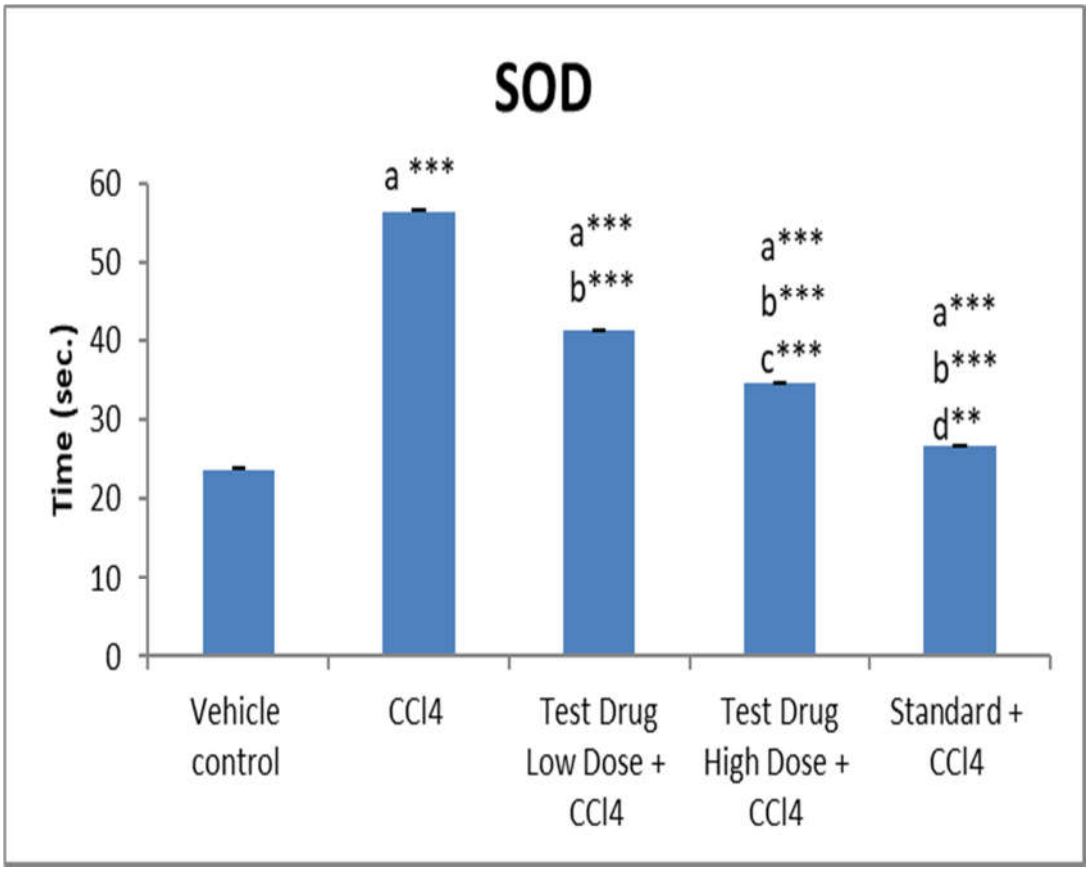

Figure 8. Graphical representation of SOD levels

One-way ANOVA showed significantly reduced SOD in the CCl4 group. Allantoin-treated groups exhibited a dose-dependent restoration of SOD levels ($P < 0.001$), with high-dose allantoin showing superior activity even compared to standard.

4. DISCUSSION

In this investigation, liver function biomarkers, antioxidant defense measures, and oxidative stress markers were evaluated in order to investigate the hepatoprotective efficacy of allantoin against carbon tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats. A well-known hepatotoxin, CCl_4 causes liver damage by producing reactive free radicals that cause oxidative stress, lipid peroxidation, and hepatocellular destruction. This study's biochemical and antioxidant changes unequivocally demonstrate that CCl_4 successfully induced hepatotoxicity.

Administration of CCl_4 resulted in a significant elevation of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels, reflecting damage to hepatocyte membranes and increased permeability leading to enzyme leakage into the circulation. Elevated ALP levels further indicate hepatobiliary dysfunction and cholestasis. Treatment with allantoin at both low and high doses significantly reduced these enzyme levels in a dose-dependent manner, demonstrating effective stabilization of hepatocellular membranes and restoration of liver function. The high-dose allantoin group showed a more pronounced reduction in enzyme levels, in some parameters exhibiting superior protection compared to the standard drug silymarin, suggesting a strong hepatoprotective efficacy.

Albumin levels were altered following CCl_4 administration, indicating disruption of hepatic synthetic function. Treatment with allantoin significantly corrected albumin levels, confirming the restoration of normal protein synthesis capacity of the liver. This improvement reflects enhanced functional recovery of hepatocytes following allantoin administration.

A major factor in CCl_4-induced liver damage is oxidative stress. lowered glutathione (GSH) and superoxide dismutase (SOD) levels were significantly lowered in the current study due to CCl_4, suggesting weakened antioxidant defense systems. Malondialdehyde (MDA), a lipid peroxidation marker, was also markedly increased at the same time, indicating widespread oxidative damage to membrane lipids. Allantoin treatment dramatically decreased MDA concentrations in a dose-dependent manner while considerably restoring GSH and SOD levels. These results imply that by boosting endogenous antioxidant defenses and preventing lipid peroxidation, allantoin successfully combats oxidative stress.

The superior antioxidant activity observed with high-dose allantoin highlights its potential role as a free radical scavenger and membrane stabilizer. The improvement in antioxidant enzyme levels and reduction in oxidative stress markers strongly correlate with the observed normalization of biochemical liver markers, reinforcing the hepatoprotective mechanism of allantoin.

Overall, the hepatoprotective effect of allantoin appears to be mediated primarily through its antioxidant properties, reduction of lipid peroxidation, and stabilization of hepatocyte membranes. The comparable—and in some cases superior—effects of allantoin relative to silymarin further emphasize its therapeutic relevance.

5. CONCLUSION

The findings of the present study clearly demonstrate that allantoin possesses significant hepatoprotective activity against carbon tetrachloride-induced liver injury in Wistar rats. Allantoin effectively attenuated CCl_4-induced elevations in liver function biomarkers, restored antioxidant enzyme levels, reduced lipid peroxidation, and improved hepatic functional integrity in a dose-dependent manner.

The hepatoprotective effects of allantoin are primarily attributed to its antioxidant and membrane-stabilizing properties, which protect hepatocytes from oxidative stress–mediated damage. The high-dose allantoin treatment exhibited pronounced protective effects, comparable to or exceeding those of the standard hepatoprotective drug silymarin.

Based on these results, allantoin may be considered a promising natural therapeutic agent for the prevention and management of liver disorders associated with oxidative stress. Further

studies are warranted to elucidate its precise molecular mechanisms and to explore its clinical applicability.

CONFLICT OF INTEREST

There were no conceivable conflicts of interest disclosed by the authors.

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