doi.org/10.36721/PJPS.2026.39.8.240.1

Comparative evaluation of *Caesalpinia bonducella* leaf extract in CCl_4-induced hepatotoxicity: Insights from lipid and protein peroxidation, DNA damage and histopathology

Samia Mushtaq[1]*, Lubna Naz[1] and Ahad Javed[2]
[1]Department of Physiology, University of Karachi, Karachi 75270, Sindh, Pakistan
[2]Department of Karachi University Business School (KUBS), University of Karachi, Karachi 75270, Sindh, Pakistan

Abstract: **Background**: *Caesalpinia bonducella* (CB) leaves, exhibiting diversified remedial impact, offering a wide range of restorative benefits, still need scientific validation due to the scarcity of reported information using in-vivo, multi-solvent-based strategies (Aqueous, Methanol, Acetone) for thorough phytochemical analysis on hepatoxicity model. As oxidative stress underlies hepatic deterioration, scientific investigations are increasingly turning to natural alternatives, especially phytochemical-based antioxidants, for the restoration of liver pathologies. **Objectives**: The current investigation was undertaken to explore the restorative potential of CB leaf extracts in CCl_4-mediated Hepatotoxicity in rats. **Methods**: Thirty-six male rats, six groups, 40-day CCl_4 treatment. Hepatotoxicity was induced with CCl_4 (0.8 ml/kg b.w.) twice weekly. The CB leaf extract groups III to V received CCl_4 with aqueous, methanolic and acetonic extracts (100mg/kg b.w., daily) respectively; however, group VI received Silymarin (25mg/kg b.w., daily). The principal biomarkers investigated included liver enzymes (ALT, AST, ALP, GGT), enzymatic antioxidants (catalase, SOD, GSH), lipid peroxidation markers (MDA, 4-HNE, 8-isoprostane), protein oxidation (carbonyls) and DNA damage indicators (8-oHdG, BPDE–DNA adducts). Histological evaluation was conducted to determine the extent of liver damage. **Results**: The acetonic extract group showed a significant reduction in AST and ALT levels, whereas the aqueous group showed comparable results for ALP and γ-GT levels. Methanolic, acetonic and aqueous extracts restored Catalase, SOD, GSH levels. The MDA and 4-HNE levels were significantly decreased in the acetonic extract group, whereas the methanolic extract group showed the significant reduction in 8-isoprostane levels compared with the CCl_4-treated group. Notably, this study is the first to report modulation of protein carbonyl, 8-oHdG and BPDE-DNA adducts by CB leaf extracts. **Conclusion**: Collectively, these findings highlight the hepatoprotective and antioxidant efficacy of *Caesalpinia bonducella* leaf extracts, with solvent-specific yet complementary mechanisms that mitigate hepatic oxidative stress, supporting their potential as natural hepatoprotective agents.

Keywords: Antioxidant activity; *Caesalpinia bonducella*; Carbon tetrachloride (CCl_4); Hepatotoxicity; Lipid peroxidation; Oxidative stress

Submitted on 10-12-2025 – Revised on 12-02-2026 – Accepted on 19-02-2026

INTRODUCTION

Hepatic disorders, particularly hepatitis, cirrhosis and hepatocellular carcinoma, represent a significant health concern, causing nearly two million fatalities per year, about 4% of total deaths (Devarbhavi *et al.*, 2023). Complete authentic and exact statistics are however not available due to many of the unreported cases. Cirrhosis alone accounts for 1.47-1.48 million deaths (Liu and Chen, 2022). Drug-induced liver injury (DILI) and Hepatitis B are leading causes of liver disease in Asia. South Asia accounts for approximately 314,000 deaths each year, placing it tenth among global regions in terms of annual mortality. DILI is a rare but serious condition, often unpredictable and linked to drug toxicity. Despite its rarity, DILI is the primary cause of acute liver failure, with fatality rates near 50% in United States (Hosack *et al.*, 2023). Approximately 21.9 percent of patients are affected especially older patients, elevated hepatic enzymes correlated with DILI, reinforcing the value of biochemical assessment in early diagnosis (Izhar *et al.*, 2025).

Caesalpinia bonducella (CB) L. (family: Caesalpiniaceae), commonly known as Fever nut, or karanjwa (local name), is a straggling, thorny, perennial shrub, with large, bi-pinnate compound leaves, yellow flowers, which are followed by green spiky pods that later become greyish and tough. These pods contain one or two shiny smooth-surfaced seeds which contain kernels. In Pakistan, the CB plant is well known for its medicinal properties and is widely distributed especially in the subtropical and temperate regions of Punjab and Sindh. CB has been used traditionally to treat various ailments. It is a popular medicinal plant in the indigenous system of medicine such as homeopathy, Ayurveda, Unani, Siddha (Ahmad *et al.*, 2023). While the pharmacological potential of CB seeds is well documented, the medicinal efficacy of its leaves remains underexplored. Although leaf extracts are known to contain saponins, tannins, flavonoids and terpenoids and have shown preliminary anticancer activity (Sasidharan *et al.*, 2021; Nithiyanandam *et al.*, 2023), systematic studies on their hepatoprotective mechanisms are still lacking.

**Corresponding author:* e-mail: samia@uok.edu.pk

As a colourless, volatile and highly stable chlorinated hydrocarbon, Carbon tetrachloride's (CCl_4) widespread use as an industrial solvent has made it a significant environmental concern. CCl_4 is a well-known hepatotoxic agent that causes hepatic necrosis, fibrosis and cirrhosis via oxidative stress. Although the demand for hepatoprotective agents is continuously increasing, the safety concerns of existing medicines present a persistent challenge, making investigations into effective medicinal strategies more promising (Singh *et al.*, 2024).

For the past few decades, much importance and focus have been given to natural products, for instance, the prevention and treatment of various human ailments using alternative green medicinal options, such as plant polyphenols and flavonoids. This paradigm shift has been attributed to the diversified benefits, low cost, easy accessibility and least side effects of those agents (Roy *et al.*, 2022). However, specific dosage, duration and safety profile of these agents must be taken into consideration. A promising protective role for flavonoids in DILI has been reported, as evidenced by modulation of specific genes involved in redox homeostasis and inflammatory pathways. The expression of cytochrome P450 enzymes is actively inhibited by these flavonoids (Yao *et al*., 2021). The scarcity of reported literature on *in-vivo*, multi-solvent-based studies has led to the conceptualization of the current study, which aims to conduct a deeper investigation into the restoration of protein and DNA damage provided by the polyphenols in leaf extracts. In fibrosis model, repeated CCl_4 exposure (4-9 weeks), consistently induces protein and DNA damage. CCl_4-derived oxidative radicals bind to proteins, lipids and nucleic acids, initiating protein oxidation, lipid peroxidation and DNA damage (Fareed *et al.*, 2024; Unsal *et al.*, 2021). Existing research on CB seed extracts has focused on hepatoprotection and antioxidant properties; however, multi-solvent-based leaf extracts have not yet been documented.

Furthermore, these studies most exclusively based on conventional liver enzymes and lipid peroxidation markers, no CB leaf extract-based studies have quantified oxidative DNA lesions 8-hydroxy-2'-deoxyguanosine (8-oHdG), Benzo[*a*]Pyrene diol epoxide (BPDE)-DNA adducts, or protein carbonyls, even though these markers are well recognized, clinically relevant markers of hepatic oxidative injury. It introduces a novel approach by evaluating solvent-specific antioxidant effects and uniquely reports modulation of 8-oHdG and BPDE-DNA adducts, markers that have not been previously studied in this context. The originality lies in validating the hepatoprotective potential of these extracts through *in-vivo* biochemical and histopathological assessments. These findings highlight the plant's promise as a lead candidate for the development of natural antioxidant-based drugs targeting liver disorders.

MATERIALS AND METHODS

Waste management and disposal

All reagents, chemicals, biological specimens, tissues and laboratory equipment were handled with strict adherence to internationally recognized safety protocols as mentioned in the guidelines provided by National Research Council (2011).

Animal procurement and acclimatization

36 Male Wistar albino rats (7 to 8 weeks old, weighing 145-180 g) were procured from the animal facility of DUHS Karachi, weighed and accommodated in decontaminated, moisture-free polypropylene cages under controlled temperature (23 ± 2 °C) and humidity (50 to 65%) with a circadian cycle (Wang *et al*., 2021). To reduce experimental variability, male rats were chosen to eliminate estrous cycle influences on biochemical and histopathological outcomes, a standard practice in CCl_4 hepatotoxicity models. A preparatory acclimatization period of one week was implemented before initiating the experimental procedures. Free access to water and food was ensured.

Preparation of leaf extracts of Caesalpinia bonducella

Leaves of CB were collected and authenticated by a taxonomist through morphological and taxonomic evaluation (Voucher No.: 100481). Standardized extraction protocols were applied and comparative evaluation demonstrated consistent results across batches, ensuring reproducibility. The leaves were rinsed, shade-dried for 7 days, powdered and stored.

Aqueous extract

The aqueous extract of *C. bonducella* leaf (CB-AQ) was prepared via the hot-water extraction method as described by Mahfoozurrahman and colleagues. 500 ml of distilled water used to suspend 100 g leaf powder. Thorough extraction of the leaf material was ensured via 8 hours of reflux per cycle, performed 3 times. Each cycle was followed by filtration with muslin cloth and Whatman (grade 1) paper. A water bath with gentle heating was used to evaporate the resultant filtrates, which were subsequently stored at 4 °C until use (Mahfoozurrahman *et al*., 2012).

Methanolic extract

Briefly, 500 mL of methanol was used to extract 100 g of powdered leaf material using a Soxhlet apparatus at 50 °C for 6 to 8 hours. The resultant extract was cooled and filtration was performed using Whatman filter paper. Rotary evaporation was performed to concentrate the filtrate, followed by storage in amber vials at 4 °C until needed (Billah *et al.*, 2013).

Acetonic extract

500 ml acetone was used to macerate 100 g of powdered leaf material at room temperature with occasional agitation for 72 hours. Sequential filtration of the mixture was performed using Whatman (grade 1) muslin cloth and filter

paper. Rotary evaporation at less than 45 °C was employed to concentrate the filtrate. The resulting extract was stored at 4 °C until needed (Billah *et al.*, 2013). Dose-dependent variability was avoided by selecting a uniform dose of 100 mg/kg, supported by published research and preliminary findings.

Experimental groups

Six experimental groups were formed through random allocation of the animals (n=6). Their body weights were checked on regular specified intervals for 40 days. All groups, except the control group, were administered CCl_4 (0.8 ml/kg b.w.) diluted 1:1 in olive oil (v/v) to rats intraperitonially, twice weekly. The experimental phase lasted for 40 days. Each group was treated according to the following protocol:

Group I: *Control*, comprised of rats that were given normal saline; *Group II: CCl_4-treated*, received CCl_4; *Group III: CCl_4+CB-AQ (Aqueous Extract)-treated Group*, received CCl_4 + 100mg extract/ kg B.W. orally via gavage per day; *Group IV: CCl_4+CB-MeOH (Methanol)-treated Group*, received CCl_4 + 100mg extract/ kg B.W. orally via gavage per day; *Group V: CCl_4+CB-ACE (Acetone)-treated Group*, received CCl_4 + 100mg extract/ kg B.W. orally via gavage per day; *Group VI: CCl_4 + Sil (Silymarin)-treated Group*, received CCl_4 + 25mg extract/ kg B.W. orally via gavage per day.

Blood and liver sample processing

After 40 days of treatment, animals were euthanized. Liver tissues were rinsed, weighed and stored at −80 °C. Blood was collected via cardiac puncture 48 hours post-final dose using heparinised and serum tubes for plasma and serum separation. Liver lobes were cleaned, weighed, fixed in 10% formalin and preserved. Homogenates were prepared by perfusing with saline and homogenizing in ice-cold 1.17% potassium chloride (Balasubramaniam *et al*., 2020; Mushtaq and Naz, 2020).

Histopathological investigation

Formalin fixation followed by paraffin embedding for making 4 µm thick sections of hepatic tissue were employed. Microscopic histopathological evaluation was done using hematoxylin and eosin. Blinded Histopathological analysis was conducted by a qualified histopathologist, blinded to treatment groups, to strengthen transparency and ensure unbiased evaluation.

Analytical procedures

ALT and AST were quantified using the (Reitman and Frankel, 1957) method. ALP by (Rec, 1972), GGT by (Szasz, 1969), with Randox kits. Antioxidants Catalase, SOD and GSH were measured as described by (Sinha, 1972), (Kono, 1978) and (Carlberg and Mannervik, 1985), respectively. Lipid peroxidation markers MDA, 4-HNE and 8-isoprostane were measured following the methods of (Ohkawa *et al*., 1979) and (Vacchiano and Tempel, 1994), respectively. Protein and DNA oxidation were assessed via carbonyls (Dalle-Donne *et al.*, 2003), 8-OHdG (Saito *et al*., 2013) and BPDE–DNA adducts (Lee *et al*., 1998) in hepatic homogenates. All spectrophotometric assays were performed using spectrophotometer (Shimadzu UV-1800, Japan) and microplate-based assays were carried out using microplate reader (Bio-Rad 680, USA).

Hepatic-lesion scoring

Hepatic injury was graded semi-quantitatively (French *et al.*, 1988). Histopathological changes were graded using a Pathologic Severity Score (0–4). A score of 0 indicated no detectable hepatocellular injury, while 1 reflected mild focal damage involving less than 25% of tissue. A score of 2 represented localized injury affecting 25–50% of the area and 3 denoted marked but regionally confined lesions. The highest score, 4, corresponded to diffuse and extensive hepatocyte necrosis throughout the tissue.

Statistical analysis

Data were processed using SPSS (v.27) and presented as mean ± SEM. Study followed a completely randomized design. Differences among groups were assessed using one-way ANOVA; intergroup differences were evaluated using the Tukey test with a $p<0.05$ significance level. For all analyses, n=6 corresponds to biological replicates, with single-run homogenate assays per sample.

RESULTS

Phytochemical screening of various leaf extracts of *Caesalpinia bonducella* revealed the presence of diverse bioactive compounds, as summarized in table 1. Alkaloids, flavonoids, tannins, saponins and glycosides were detected in differing proportions across the extracts, highlighting the plant's rich chemical profile and potential pharmacological significance. Comparable reduction was shown in body weight across all extract groups compared with Group II. Significant amelioration ($p<0.05$) was observed in body weight compared with Group II, suggesting the restorative potential of the methanolic extract in ameliorating pathological alterations within the body. Group VI exhibited a statistically significant decrease in body weight ($p < 0.01$) relative to Group II. Significantly reduced absolute liver weight among all the groups relative to Group II was observed, indicating tissue damage restoration (Table 2) provided by these extracts' antioxidative potential. The extracts group restored the devastating effects of CCl_4, supported by the histopathological findings (Fig. 1) and further confirmed by improved liver enzymes (Table 3).

Table 3 depicts hepatic enzyme levels among experimental groups. Significantly elevated ALT levels of Group II indicate hepatic deterioration caused by CCl_4. The extracts in Groups III and V have shown a significant reduction in ALT levels compared to Group II ($p < 0.001$). However, the greatest restoration of CCl_4-mediated deterioration was observed in Group V.

Table 1: Phytochemicals in various leaf extracts of *Caesalpinia bonducella.*

Extract type	Flavonoids	Tannins	Saponins	Coumarins	Steroids	Terpenoids	Glycosides	Alkaloids
Aqueous extract	+	+	+	-	-	-	+	-
Methanolic extract	+	-	+	-	+	+	+	+
Acetonic extract	+	-	+	-	+	+	-	+

Table 2: Body and Hepatic Weight Analysis among the Experimental Groups; Control group, CCl_4 treated group, CCl_4 with Aqueous Leaf Extract, CCl_4 with Methanolic Leaf Extract, CCl_4 with Acetonic Leaf Extract and CCl_4 with Silymarin Supplementation.

Groups (n=6)	Body and hepatic weight analysis			
	Baseline body weight (g)	Terminal body weight (g)	Absolute liver weight (g)	Normalised liver weight (%)
I-Control	143.2±2.57	201.67±4.514	5.33±0.147	2.64±0.065
II-CCl_4	189±4.573^c	186.3±6.839^n	6.65±0.256^c	3.57±0.088^c
III-CCl_4+AQ	168.83±6.925^a	170.8±7.368^n	5.73±0.206^c	3.365±0.076^n
IV-CCl_4+MeoH	163.83±6.384^b	159.83±6.848^a	5.48±0.142^c	3.46±0.167^n
V-CCl_4+ACE	165.83±8.749^b	177.67±9.89^n	6.05±0.133^a	3.44±0.144^n
VI-CCl_4+SIL	173.5±7.482^n	156.67±9.559^b	5.65±0.329^b	3.61±0.0295^n

Mean ± SEM values are reported. Superscripts indicate statistical significance: a ($p<0.05$), b ($p<0.01$), c ($p<0.001$), d ($p < 0.000001$); n denotes non-significant differences ($p>0.05$).

Table 3: Hepatic tissue homogenate enzyme-levels among the experimental groups; Control group, CCl_4 treated group, CCl_4 with Aqueous Leaf Extract, CCl_4 with Methanolic Leaf Extract, CCl_4 with Acetonic Leaf Extract and CCl_4 with Silymarin Supplementation.

Groups (n=6)	Hepatic tissue homogenate enzyme-levels			
	ALT U/g	AST U/g	ALP U/g	γ-GT U/g
I-Control	13.13±0.439	17.48±0.44	120.22±0.68	19.6±0.34
II-CCl_4	20.67±0.87^c	22.4±0.811^a	132.8±1.34^c	23.2±0.909^b
III-CCl_4+AQ	11.8±0.364^c	16.07±0.72^b	109±0.27^c	14.12±0.309^c
IV-CCl_4+MeoH	15.75±0.528^b	20.95±0.56^n	131.75±0.99^n	16.03±0.398^c
V-CCl_4+ACE	7.06±0.326^c	12.12±0.63^c	128.7±0.93^n	14.67±0.486^c
VI-CCl_4+SIL	9.42±1.7^c	16.6±0.704^a	106.9±1.008^c	18.05±0.661^c
Effect size (Eta-Squared)	0.829	0.865	0.982	0.858

Mean ± SEM values are reported. Superscripts indicate statistical significance: a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$), d ($p < 0.000001$); n denotes non-significant differences ($p > 0.05$). Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (γGT).

Table 4: Hepatic tissue homogenate antioxidant enzyme-levels among the experimental groups; Control group, CCl_4 treated group, CCl_4 with Aqueous Leaf Extract, CCl_4 with Methanolic Leaf Extract, CCl_4 with Acetonic Leaf Extract and CCl_4 with Silymarin Supplementation.

Groups (n=6)	Hepatic tissue homogenate antioxidant enzyme levels		
	Catalase (μ mol/g)	SOD (μ mol/g)	GSH (μ mol/g)
I-Control	19.78±0.46	111.3±2.17	1.18±0.0409
II-CCl_4	7.416±0.318^c	34.91±1.79^c	0.44±0.004^c
III-CCl_4+AQ	15.39±0.556^c	90.26±0.435^c	1.9±0.0116^c
IV-CCl_4+MeoH	20.63±0.497^c	142.83±1.09^c	1.12±0.0036^c
V-CCl_4+ACE	12.89±0.487^c	186.58±0.95^c	0.59±0.0087^c
VI-CCl_4+SIL	21.53±0.789^c	200.98±1.428^c	1.16±0.0091^c
Effect size (Eta-squared)	0.94	0.99	0.99

Mean ± SEM values are reported. Superscripts indicate statistical significance: a ($p<0.05$), b ($p<0.01$), c ($p < 0.001$), d ($p < 0.000001$); n denotes non-significant differences ($p>0.05$). SOD: Superoxide Dismutase; GSH: Glutathione.

Table 5: Hepatic tissue homogenate lipid peroxidation marker levels among the experimental groups; Control group, CCl_4 treated group, CCl_4 with Aqueous Leaf Extract, CCl_4 with Methanolic Leaf Extract, CCl_4 with Acetonic Leaf Extract and CCl_4 with Silymarin Supplementation.

Groups (n=6)	Lipid peroxidation markers		
	MDA (µmol/g)	4-HNE (pg/ml)	8-Isoprostane (ng/L)
I-Control	0.933±0.042	19.55±0.48	14.67±0.61
II-CCl_4	2.27±0.558^c	22.6±0.608^b	219.5±1.024^c
III-CCl_4+AQ	1.62±0.030^c	17.97±0.667^c	105±1.291^c
IV-CCl_4+MeoH	2.0±0.0516^b	18.31±0.366^c	54.33±0.988^d
V-CCl_4+ACE	0.7±0.036^c	14.58±0.349^c	138.8±0.83^b
VI-CCl_4+SIL	1.23±0.055^c	18.21±0.44^c	15.33±0.49^c
Effect size (Eta-squared)	0.968	0.968	0.99

Mean ± SEM values are reported. a ($p<0.05$), b ($p<0.01$), c ($p < 0.001$), d ($p< 0.000001$); n denotes non-significant differences ($p>0.05$). MDA: Malonaldehyde; 4-HNE: 4-Hydroxynonenal.

Table 6: Hepatic tissue homogenate protein peroxidation marker levels among the experimental groups; Control group, CCl_4 treated group, CCl_4 with Aqueous Leaf Extract, CCl_4 with Methanolic Leaf Extract, CCl_4 with Acetonic Leaf Extract and CCl_4 with Silymarin Supplementation.

Groups (n=6)	Protein peroxidation marker
	Protein carbonyl (nmol/g)
I-Control	0.76±0.005
II-CCl_4	1.34±0.16^c
III-CCl_4+AQ	1.16±0.0408^n
IV-CCl_4+MeoH	1.19±0.046^n
V-CCl_4+ACE	0.85±0.008^b
VI-CCl_4+SIL	1.146±0.044^n
Effect size (Eta-squared)	0.636

Mean ± SEM values are reported. a ($p<0.05$), b ($p<0.01$), c ($p< 0.001$), d ($p< 0.000001$); n denotes non-significant differences ($p>0.05$).

Table 7: Hepatic tissue homogenate oxidative DNA damage by-products among the experimental groups; Control group, CCl_4 treated group, CCl_4 with Aqueous Leaf Extract, CCl_4 with Methanolic Leaf Extract, CCl_4 with Acetonic Leaf Extract and CCl_4 with Silymarin Supplementation.

Groups (n=6)	Oxidative DNA damage by-products	
	8-oHdG (ng/g)	BPDE-DNA Adduct (ng/g)
I-Control	1.82±0.029	0.337±0.0084
II-CCl_4	4.27±0.024^c	1.184±0.0336^c
III-CCl_4+AQ	3.63±0.038^c	0.46±0.0083^c
IV-CCl_4+MeoH	1.99±0.049^c	0.76±0.0059^c
V-CCl_4+ACE	0.99±0.0147^c	1.105±0.03^a
VI-CCl_4+SIL	0.92±0.0226^c	0.886±0.0024^b
Effect size (Eta-squared)	0.995	0.981

Mean ± SEM values are reported. a ($p<0.05$), b ($p<0.01$), c ($p<0.001$), d ($p<0.000001$); n denotes non-significant differences ($p>0.05$).8oHdG: 8-hydroxy-2′-deoxyguanosine; BPDE: Benzo[a]pyrene Diol Epoxide

The AST, ALP and γ-GT levels in Group II are significantly elevated compared with Group I, suggesting CCl_4 toxicity. Group V has shown the greatest significant reduction ($p<0.001$) in AST levels compared with Group II. A significant reduction in γ-GT levels was observed across all three extract groups compared with Group II ($p<0.001$). However, Group III exhibited the most substantial decrease in γ-GT levels among the treatment groups, followed by Group V (CCl_4+Acetonic Extract Group), compared with Group II (CCl_4-treated Group), suggesting the potential benefits of these extracts in ameliorating Hepatotoxicity. CCl_4-mediated oxidative damage was evident by significantly reduced levels of Catalase, SOD and GSH ($p<0.001$) of Group II as compared to Group I (Table 4). Mitigation of Hepatotoxicity was revealed by significant elevations in antioxidants in the extract groups, with the highest restorative potential shown in Catalase levels in Group IV, SOD in Group V and GSH in Group III ($p<0.001$) compared with Group II.

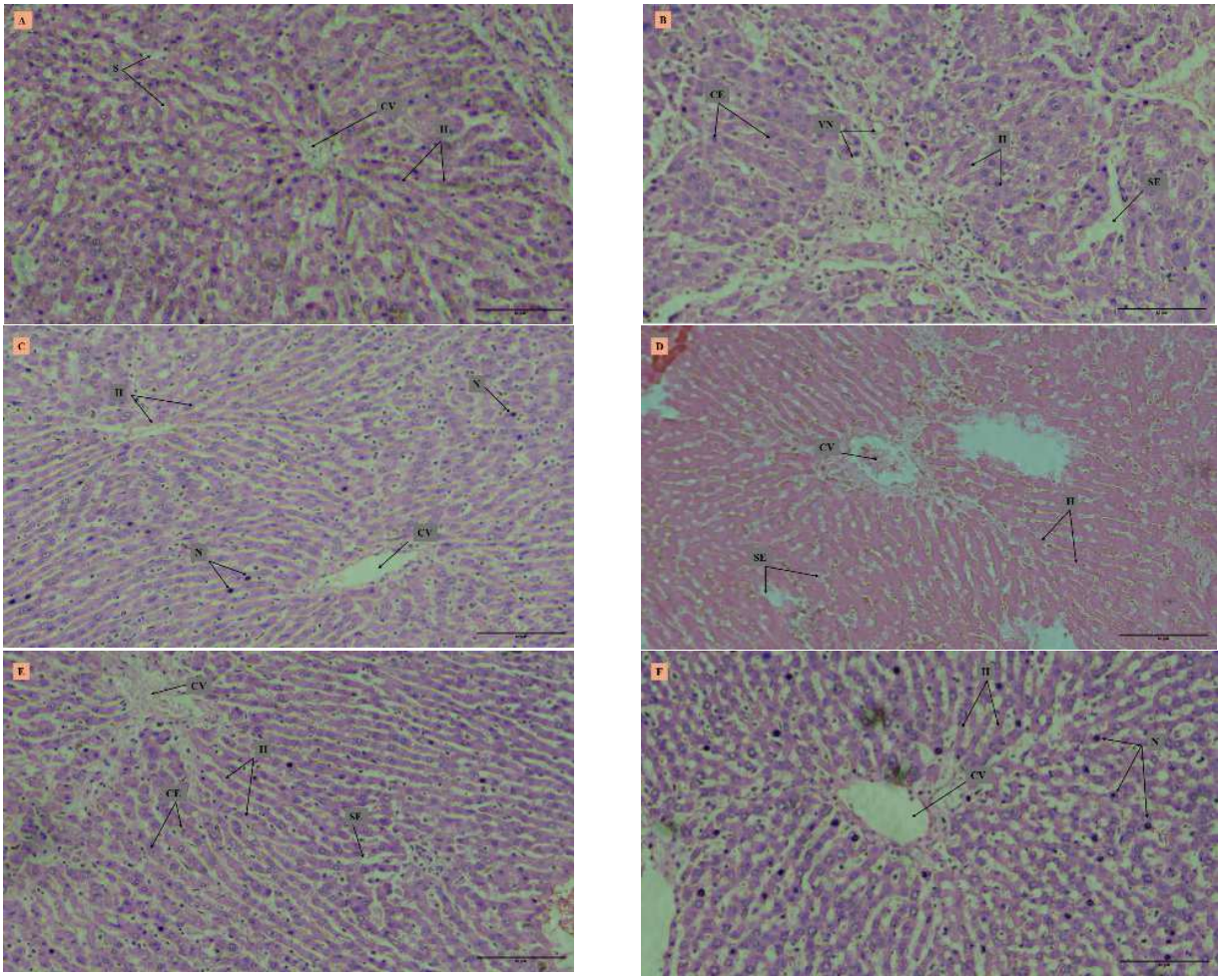

Fig 1: Representative photomicrographs of hepatic sections stained with H&E (20x Magnification), illustrating centrilobular and periportal architecture depicting the Effects of various extracts of *Caesalpinia bonducella* Leaf on CCl_4-induced liver injury.
(a) Control group hepatic tissue exhibited normal hepatocyte architecture with mild sinusoidal fenestration, lacking portal inflammation, sinusoidal dilation, or hepatocellular degeneration. H: Hepatocytic Strings; CV: Central Vein; S: Sinusoid; (b) CCl_4-treated liver showed disrupted hepatocyte architecture, marked inflammation, steatosis, nuclear enlargement, vesicular nuclei and extensive sinusoidal dilation with degenerative changes. H: Disorganized Hepatocytic cords with degenerative hepatocytes; VN: Vesicular Nuclei; CE: Cellular Enlargement; SE: Sinusoidal Expansion; (c) CCl_4 + aqueous extract-treated liver showed mild portal inflammation, lobulitis, minimal degeneration and preserved hepatocytic architecture without steatosis or fibrosis. H: Hepatocytic strings; CV: Central Vein; N: Scattered Neutrophils; (d) CCl_4 + methanolic extract-treated liver showed portal inflammation, vein congestion, mild lobulitis, degeneration and preserved hepatocytic cord architecture without steatosis or fibrosis. H: Hepatocytic strings; CV: Central Vein; SE: Sinusoidal Expansion. € CCl_4 + acetonic extract-treated liver showed mild inflammation, lobulitis, vein congestion, focal degeneration and preserved hepatocytic cord architecture without steatosis or fibrosis. H: Hepatocytic strings; CV: Central Vein; SE: Sinusoidal Expansion; CE: Cellular Enlargement; (f) CCl_4 + Silymarin-treated liver showed mild inflammation, minimal congestion, no degeneration, steatosis, or fibrosis. H: Hepatocytic strings; CV: Central Vein; N: Neutrophils.

MDA, 4-HNE and 8-isoprostane are the best byproducts of lipid peroxidation. Oxidative damage to polyunsaturated fatty acids (PUFAs) leads to lipid radical formation, which generates MDA, 4-HNE and 8-isoprostane through subsequent reactions involving oxygen and lipid hydroperoxides. (Atalay Ekiner *et al.*, 2024). A marked elevation in lipid peroxidation markers in Group II as compared to Group I, was suggestive of the detrimental effects of CCl_4. Although significant reductions in MDA, 4-HNE and 8-isoprostane levels were observed in the extract groups, considerable reductions were seen in MDA (Group V, $p < 0.001$) and 4-HNE (Group V, $p<0.001$) levels and in 8-isoprostane levels (Group IV, $p < 0.0001$) compared with Group II (Table 5), endorsing the remedial potential of these extracts.

Table 8: Hepatic histo-pathologically noticed characteristics among the experimental groups; Control group, CCl_4 treated group, CCl_4 with Aqueous Leaf Extract, CCl_4 with Methanolic Leaf Extract, CCl_4 with Acetonic Leaf Extract and CCl_4 with Silymarin Supplementation.

Histopathological findings	Control	CCl_4	CCl_4+AQ	CCl_4+MeoH	CCl_4+ACE	CCl_4+Sil
Portal inflammation	0	2	1	1	1	1
Hydroptic degeneration	0	0	0	0	0	-
Cellular enlargement	0	3	1	1	1	-
Fatty change	0	2	0	0	0	-
Portal fibrosis	0	0	0	0	0	-
Periportal fibrosis	0	0	0	0	0	-
Focal lobulitis	0	3	1	0	1	1
Total score	0	10	3	2	3	2
Sinusoidal expansion	-	+	+	-	+	-
Decayed hepatic cells	-	+++	+	+	+	-
Ballooning degeneration	-	++	-	-	-	-
Vesicular nuclei	-	+++	-	-	-	-

Pathologic Severity Scores: 0, no histologically detectable hepatocellular injury; 1, mild focal damage (< 25% area, localized injury (25 to 50% area); 3, marked but regionally confined lesions; 4, diffuse and extensive necrosis - = Absent, + = Mild, ++ = Moderate, +++ = severe.

Protein carbonylation is a stable marker of oxidative stress, formed by the addition of carbonyl groups toamino acids through oxidation or glycation (Dalle-Donne *et al*., 2003).

Significant elevation of protein carbonyl levels in Group II was observed as compared to Group I ($p<0.001$) (Table 6). However, relative to Group II, the protein oxidative marker levels in Group V were significantly lower, suggesting the remedial potential of the extract in mitigating the deleterious effects of CCl_4.

8-OHdG and BPDE-DNA adducts are distinct DNA damage markers: 8-OHdG reflects oxidative stress, while BPDE-DNA adducts result from metabolic activation of carcinogenic PAHs like benzo[a]pyrene (Bukowska and Duchnowicz, 2022). The 8-oHdG and BPDE-DNA adduct levels in Group II were significantly elevated ($p<0.001$) as compared to Group I, indicating pronounced oxidative damage owing to CCl_4 administration. The extracts groups, however showed significant reduction ($p<0.001$) in 8-oHdG and BPDE-DNA adduct levels when compared with the Group II, with Group V exhibiting the highest therapeutic efficacy in 8-oHdG levels and Group III and IV in BPDE-DNA adduct levels ($p<0.001$) in comparison with Group II, ameliorating oxidative stress (Table 7).

Histopathological evaluation of liver tissues revealed marked differences among the experimental groups, as summarized in table 8. The CCl_4-treated group showed severe pathological changes, including portal inflammation, fatty change, focal lobulitis and extensive cellular damage. In contrast, groups receiving *Caesalpinia bonducella* leaf extracts (aqueous, methanolic and acetonic) or silymarin supplementation exhibited notably reduced scores, with preservation of hepatic architecture and attenuation of degenerative changes.

DISCUSSION

Assessment of body weight is a valuable measure of systemic well-being, with variations often indicating metabolic disruption, stress, or treatment-induced toxicity. Weight loss in group II was attributed to the deteriorating effects of CCl_4 on the body and the liver (Table 2). Furthermore, CCl_4 administration causes anorexia leading to reduced appetite. A study showed that administering a low dose of CCl_4 to rats led to anorexia regardless of hepatic injury or dietary restriction (Okamoto and Okabe, 2000), which might explain why rats had reduced appetite and therefore a marked reduction in body weight. However, as food intake was not monitored in the study, it has been acknowledged as a limitation and aim to include it in future studies. The reduction in body weight observed in extract-treated rats may be attributed to bioactive constituents that modulate lipid metabolism, potentially by inhibiting lipogenesis or altering other metabolic pathways. When combined with the metabolic disturbances induced by CCl_4, these effects may synergistically exacerbate weight loss, independent of hepatic injury. A substantial body of literature supports comparable results (Shammah and Thenmozhi, 2023). Alterations in organ weight are widely acknowledged as reliable indicators in studies involving repeated exposure to toxic substances. In contrast, normalised liver weight helps determine if liver weight changes are due to chemical effects or just body weight variation. Changes in hepatic weight may reflect tissue-level effects, whether due to damage or natural adaptation, as evidenced by variations in absolute or normalised liver weight (Mezencev *et al.*, 2024).

Alterations in liver enzyme levels best show hepatocellular integrity; the results presented in table 3 highlight the extent of hepatic deterioration and the therapeutic

relevance of the CB leaf extracts. Severe hepatic injury was evident via marked increase in liver enzymes in Group II as compared to Group I. Especially, Group V caused significant amelioration of AST, ALT, whereas the aqueous treated group leads to marked reduction in ALP levels. Comparable findings have been reported by other study (Sumalatha *et al*., 2016), who noted significant enzyme normalization and improved tissue architecture in a similar experimental setup. A direct comparison of CB leaf extracts' ability to ameliorate γ-GT levels among the experimental groups cannot be conducted due to the absence of direct evidence, highlighting the need for further focused investigation.

The antioxidant profile presented in table 4 provides a clear indication of the extent of oxidative damage caused by CCl_4 and the protective role of the extracts in mitigating its deleterious effects. Providing the major frontline defensive role with the highest enzymatic capability, catalase plays far beyond its widely accepted role of neutralizing hydrogen peroxide and preventing consequent oxidative deterioration, as evidenced by recent studies. Its dynamic shuttling between subcellular compartments (cytosol and nucleus) enables it to deeply involve in redox signalling, thereby influencing cell fate (survival/apoptosis) (Anwar *et al.*, 2024). The toxic effects induced by CCl_4 were effectively mitigated by the methanolic and Silymarin Groups, with significant elevations in tissue catalase levels (Table 4), indicating the restorative potential of these extracts. Beyond GSH's role in preventing lipid peroxidation, it also contributes to cell fate, signal transduction and redox regulation. Aqueous extract showed the highest efficacy in restoring GSH levels and the second-highest efficacy for catalase, which might be attributable to the cassane diterpenes, flavonoids, saponins and tannins in the extract, which have strong radical-scavenging effects. The methanolic and acetonic groups exhibited comparable restoration of SOD levels owing to their phenolic and triterpenoid content. Comparable findings were reported by other studies with paracetamol (Gupta and Kumar, 2003) and nicotine-induced (Bharath *et al.*, 2018) hepatic deterioration.

The extent of lipid peroxidation is well reflected via MDA. 4-HNE and 8-isoprostane levels. Comparable reduction in lipid peroxidation markers in all extract groups was observed, however, acetonic extract containing terpenoids, sterols and flavonoids possess the highest restorative potential for reducing MDA and 4-HNE levels possibly via hepatic membrane stabilization, inhibition of CYP-450 mediated CCl_4 bioactivation and reduction in lipid peroxidation markers, whereas methanolic extract with intermediate polarity mainly containing flavonoids, alkaloids and terpenoids, showed the highest restoration of 8-isoprostane levels (Table 5) might be due to the antioxidant, membrane stabilization effects that lead to consequent reduction in lipid peroxidation. Owing to the lack of existing studies on the direct measurement of 4-HNE and 8-isoprostane levels with the effect of CB leaf, there is a gap in the research literature and direct comparison cannot be made. However, comparable findings on MDA reduction via CB leaf extract (Gupta *et al.*, 2005) suggest a decline in the other two markers as well, because all arise from similar processes.

Serving as stable and robust marker of protein peroxidation, protein carbonyl levels directly reflect oxidative damage to cellular proteins. All three extract groups caused amelioration of CCl_4-induced hepatic damage by restoring the protein carbonyl levels, with acetonic group containing lipophilic compounds (terpenoids, sterols, flavonoids) exhibiting highest remedial impact (Table 6), which might be attributable to protection against membrane disruption and modulation of CCl_4 metabolism and consequent-reduction of oxidative degradation of proteins. Direct evidence of protein carbonyl reduction via CB leaf extract is lacking in the literature, precluding direct comparison.

Formation of 8-oHdG and BPDE-DNA adducts occur via different pathways, with oxidative stress serving as the major cause of 8-oHdG generation, whereas metabolism of carcinogenic PAHs such as benzo[a]pyrene's leads to its conversion into BPDE, that sequentially binds to DNA. BPDE, a metabolite of polycyclic aromatic hydrocarbons, forms bulky DNA adducts through covalent binding, whereas CCl_4 undergoes bioactivation via cytochrome P450 enzymes, generating trichloromethyl radicals that induce oxidative stress and lipid peroxidation, leading to indirect DNA damage. Table 7 exhibits comparable increment in 8-oHdG and BPDE-DNA adduct levels in CCl_4 group, suggesting profound oxidative and genotoxic stress, which was efficiently restored via the CB leaf extract groups with acetonic group showing the highest therapeutic efficacy in ameliorating 8-oHdG levels and aqueous group exhibiting the highest remedial impact on BPDE-DNA adduct reduction demonstrating significant antigenotoxic protection. Since the current investigation is the first to evaluate these biomarkers in relation to CB leaf extracts, the findings provide novel insights to their therapeutic modulation and establish a foundation for future comparative research.

CONCLUSION

CB exhibit extensive therapeutic attributes, however its validation via in-vivo multi-solvent-based leaf extractions remains under-reported. Different CB leaf extracts were investigated for their hepatoprotective potential in CCl_4-mediated toxicity. Briefly, acetonic preparation significantly reduced liver transaminases, MDA and 4-HNE; aqueous extract mitigates ALP and γ-GT. 8-isoprostane was improved substantially by methanolic extract and antioxidant enzymes were improved by all

three extracts. Given its preclinical status and the specific extract types employed, further investigations including pharmacokinetics, toxicology and extract standardization are required to establish translational relevance. The current findings suggest that CB leaf extract exhibits hepatoprotective potential, providing first comparative evidence of multi-solvent extracts against CCl_4-induced hepatotoxicity and establishing a basis for future therapeutic exploration.

Acknowledgement
The authors sincerely acknowledge the assistance and facilities provided by the Department of Physiology, University of Karachi, for conducting the study.

Authors' contributions
SM and LN: Designed the study framework; SM: Carried out experiments and data interpretation; SM and AJ: Data collection and statistical assistance. All three authors critically reviewed the final version of the manuscript.

Funding
There was no funding.

Data availability statement
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethical approval
The study protocol was approved by the Institutional Bioethics Committee of the University of Karachi (ID: IBC KU-231-A). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declare no conflict of interest.

Supplementary data
https://www.pjps.pk/uploads/2026/06/SUP1780913541.pdf

REFERENCES

Ahmad K, Quamari M, Ahmad H and Hafiz KA (2023). Phytochemical profile and pharmacological activities of karanjawa (*Caesalpinia bonducella* L.): An important botanical origin drug of Unani System of Medicine. *RGUSH J AYUSH Sci,* **10**(1):1-13.

Anwar S, Alrumaihi F, Sarwar T, Babiker A, Khan A, Prabhu S and Rahmani A (2024). Exploring therapeutic potential of catalase: Strategies in disease prevention and management. *Biomol,* **14**(6): 1-31.

Atalay Ekiner S, Gęgotek A and Skrzydlewska E (2024). Inflammasome activity regulation by PUFA metabolites. *Front Immunol,* **15**: 1-23.

Balasubramaniam G, Sekar M, Varadarajan M and Badami S (2020). Antioxidant and hepatoprotective activities of strobilanthes kunthianus against carbon tetrachloride-induced hepatotoxicity in rats. *Pharmacogn J,* **12**(5): 1143-51.

Bharath K, Senthilkumar S, Kayalvizhi E, Ponnulakshmi R, Selvaraj J, Vishnupriya V, Shyamaladevi B and Madhan K (2018). A study on the nephro protective effect of *Caesalpinia bonducella* on nicotine-induced rats. *Drug Inven Today,* **10** (4): 3512-3519.

Billah MM, Islam R, Khatun H, Parvin S, Islam E, Islam SA and Mia AA (2013). Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of *Caesalpinia bonducella* (L.) Roxb leaves. *BMC Complement Altern Med,* **13**(1): 1-7.

Bukowska B and Duchnowicz P (2022). Molecular mechanisms of action of selected substances involved in the reduction of benzo [a] pyrene-induced oxidative stress. *Molecules,* **27**(4):1-18.

Carlberg I and Mannervik B (1985). [59] Glutathione reductase. *Methods Enzymol*, **113**:484-490.

Dalle-Donne I, Rossi R, Giustarini D, Milzani A, and Colombo R (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta*, **329**(1-2):23-38.

Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E and Kamath PS (2023). Global burden of liver disease: 2023 update. *J Hepatol,* **79**(2):516-537.

Estévez M, Díaz-Velasco S and Martínez R (2022). Protein carbonylation in food and nutrition: A concise update. *Amino Acids,* **54**(4):559-573.

Fareed MM, Khalid H, Khalid S and Shityakov S (2024). Deciphering molecular mechanisms of carbon tetrachloride-induced hepatotoxicity: A brief systematic review. *Curr Mol Med,* **24** (9): 1124-1134.

French S, Miyamoto K, Ohta Y and Geoffrion Y (1988). Pathogenesis of experimental alcoholic liver disease in the rat. *Methods Achiev Exp Pathol,* **13**:181-207.

Gupta M and Kumar RS (2003). Hepatoprotective effects and antioxidant role of *Caesalpinia bonducella* on paracetamol-induced hepatic damage in rats. *Nat Prod Sci,* **9**(3):186-191.

Gupta M, Mazumder U, Kumar S, Sivakumar T, Gomathi P and Rajeshwar Y (2005). Antioxidant defense system induced by a methanol extract of *Caesalpinia bonducella* in rat liver. *Pharm Biol,* **43**(5): 411-419.

Hosack T, Damry D and Biswas S (2023). Drug-induced liver injury: A comprehensive review. *Therap Adv Gastroenterol,* **16**: 1-13.

Izhar H, Naz Z, Amanat A, Khan MN, Khorasgani MA, Noushad A, Hamid A and Zanoor L (2025). Comprehensive assessment of drug-induced liver injury (DILI) among patients receiving polypharmacy in tertiary care settings: A clinical and biochemical evaluation. *IJBR,* **3**(11): 122-128.

Kono Y (1978). Generation of superoxide radical during autoxidation of hydroxylamine and an assay for

superoxide dismutase. *Arch Biochem Biophys,* **186**(1): 189-195.

Lee BM, Jang JJ and Kim HS (1998). Benzo [a] pyrene diol-epoxide-I-DNA and oxidative DNA adducts associated with gastric adenocarcinoma. *Cancer Lett,* **125**(1-2): 61-68.

Liu YB and Chen MK (2022). Epidemiology of liver cirrhosis and associated complications: Current knowledge and future directions. *World J Gastroenterol,* **28**(41): 5910-5930.

Mahfoozurrahman M, Hifzulkabir Asif M and Ansari JA (2012). Analgesic and anti-inflammatory activity of aqueous extract of *Caesalpinia bonducella* leaf in experimental animal model. *Int J PharmTech Res,* **4**(2): 97-103.

Mezencev R, Feshuk M, Kolaczkowski L, Peterson GC, Zhao QJ, Watford S and Weaver JA (2024). The association between histopathologic effects and liver weight changes induced in mice and rats by chemical exposures: An analysis of the data from Toxicity Reference Database (ToxRefDB). *Toxicol Sci,* **200**(2): 404-413.

Mushtaq S and Naz L (2020). Curative potential of quercetin against carbon tetrachloride-induced hepatotoxicity in Wistar rats. *J Anim Plant Sci,* **30**(6):1408-1414.

Nithiyanandam S, Jaisankar V, Parthasarathy M, Katturajan R and Prince SE (2023). Antioxidant mediated defensive potency of *Caesalpinia bonducella* nut on acetaminophen-inebriated spleen and cardiotoxicity: Implications on oxidative stress and tissue morphology in an *in-vivo* model. *Indian J Biochem Biophys,* **60**(4): 297-306.

Ohkawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem,* **95**(2): 351-358.

Okamoto T and Okabe S (2000). Carbon tetrachloride treatment induces anorexia independently of hepatitis in rats. *Int J Mol Med,* **6**(2): 181-184.

Rec G (1972). Colorimetric method for serum alkaline phosphatase determination. *J Clin Biochem,* **10**(2): 182-184.

Reitman S and Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol,* **28**(1): 56-63.

Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, Alshahrani MY, Islam S and Islam MR (2022). Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *BioMed Res Int,* **2022**(1): 1-9.

Saito K, Aoki H, Fujiwara N, Goto M, Tomiyama C and Iwasa Y (2013). Association of urinary 8-OHdG with lifestyle and body composition in elderly natural disaster victims living in emergency temporary housing. *Environ health prev med,* **18**(1): 72-77.

Sasidharan S, Srinivasa Kumar K, Kanti Das S and Hareendran Nair J (2021). Caesalpinia bonduc: A ubiquitous yet remarkable tropical plant owing various promising pharmacological and medicinal properties with special references to the seed. *Med Aromat Plants,* **10**(7): 1-19.

Shammah CJ and Thenmozhi A (2023). Anti-atherogenic activity of ethyl acetate and ethanolic extracts of *caesalpinia bonducella* linn. f. on high-fat diet-induced atherosclerosis in rats. *Asian J Pharm Clin Res,* **16**(8): 111-115.

Singh D, Khan MA and Siddique HR (2024). Unveiling the therapeutic promise of natural products in alleviating drug-induced liver injury: Present advancements and future prospects. *Phytother Res,* **38**(1): 22-41.

Sinha AK (1972). Colorimetric assay of catalase. *Anal Biochem,* **47**(2): 389-394.

Sumalatha S, Padma D, Pai KSR, Kotian SR, Kumar N and Bhat KM (2016). Hepatoprotective activity of aqueous extract of *Caesalpinia bonduc* against CCl4 induced chronic hepatotoxicity. *Int J Pharm Pharm Sci,* **8**(4): 207-211.

Szasz G (1969). A kinetic photometric method for serum γ-glutamyl transpeptidase. *Clin Chem,* **15**(2): 124-136.

Unsal V, Cicek M and Sabancilar I (2021). Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Rev Environ Health,* **36**(2): 279-295.

Vacchiano CA and Tempel GE (1994). Role of nonenzymatically generated prostanoid, 8-iso-PGF2 alpha, in pulmonary oxygen toxicity. *J Appl Physiol,* **77**(6): 2912-2917.

Wang YJ, Xu HY, Huo XH, Fan SE and Nabi XH, (2021). Multi-stress accelerated liver cancer progression in rats treated with diethylnitrosamine. *Chin Med J*, **134**(14): 1762-1764.

Yao L, Nisar MF, Yan T and Wan C (2021). Potential effects of dietary isoflavones on drug-induced liver injury. *J Food Qual,* **2021**(1): 1-10.