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Protective Effects of Methanolic Extract of *Telosma cordata* against Aceclofenac Induced Hepatotoxicity in Rats

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Abstract

Telosma cordata is traditionally used as an effective agent for treating conjunctivitis and back pain but has not been thoroughly studied. In current study, the hepatoprotective aptitude of *T. cordata* was evaluated against Aceclofenac (ACF) instigated oxidative stress in rats. Leaves of *T. cordata* were extracted in methanol and various fractions were prepared by polarity-based method. Qualitative and quantitative spectrophotometric analysis along with HPLC-DAD analysis was performed on samples. Antioxidant potential of extracts was identified by DPPH method while BSA method was used to assess the anti-inflammatory activity. Rats were divided into various groups and oxidative stress was induced by ACF in liver of rats. *T. cordata* Methanol extract (TCM) was used to mitigate the oxidative stress. Serum analysis for liver enzymes and tissue analysis for enzymatic and non-enzymatic antioxidants was performed. Qualitative and quantitative spectrophotometric analysis of various extracts depicted the presence of phenolics, flavonoids, and tannins. *In vitro* antioxidant and anti-inflammatory studies further elaborated the protective potential of plant extracts. Furthermore, treatment of rats with ACF showed decline ($p<0.05$) in the level of liver antioxidant enzymes, in contrast, elevation was found in the concentration of hydrogen peroxide and nitrite in hepatic tissues. Increased serum markers of the liver were revealed after ACF treatment. Hepatocyte inflammation and injuries were prominent in histopathological studies of the liver in ACF-treated rats. However, the administration of TCM to ACF-intoxicated rats reduced liver injuries and restored the levels of antioxidant enzymes and free radical towards normal rats. *T. cordata* possess strong antioxidant and anti-inflammatory potential due to presence of polyphenols and can help in development of drug against liver injuries with strong antioxidant potential.

Keywords: *Telosma cordata*; Antioxidants; Anti-inflammatory; Oxidative stress; Aceclofenac

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Abbreviations

TCM: *Telosma cordata* Methanol extract; ER: Endoplasmic Reticulum; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Tannin Content; HPLC: High Performance Liquid Chromatography; DPPH: 2,2-Diphenylpicrylhydrazyl; BSA: Bovine Serum albumin; ACF: Aceclofenac; TLC: Total Leukocyte Count; MCH: Mean Corpuscular Hemoglobin; PTC: Platelets Count; MCV: Mean Corpuscular Volume; ALT: Alanine Transferase; AST: Aspartate Transaminase; ALP: Alkaline Phosphate; SOD: Superoxide Dismutase; POD: peroxidase; CAT: Catalase

Introduction

Oxidative stress is illustrated as over production of Reactive Oxygen Species (ROS), resulting in injury to molecules like proteins, lipids, and DNA [1]. ROS have the ability to initiate lipid peroxidation, known as a chain reaction, and cause oxidation of other cellular components, such as DNA and proteins. The main cause of liver ailment is lipid peroxidation which arises due to prolonged exposure to hepatotoxins. These free radicals enhance the production of Thiobarbituric Reactive Substances (TBARS), when they interact with the polyunsaturated fatty acids, hence leading to necrosis. Therefore, Oxidative stress causes several ailments i.e., cancer, atherosclerosis, nervous disorder, diabetes, idiopathic pulmonary fibrosis, asthma, and liver fibrosis. General metabolic reactions and some biological factors including air containments aid in the production of ROS in living organisms [2-4].

Aceclofenac (ACF); a Non-Steroidal Anti-Inflammatory Drug (NSAID), is a well-known orally administered phenylacetic acid derivative that has been reported as potential drug for treatment of certain conditions like osteoarthritis [5]. Injury to the gastrointestinal mucosa, bleeding peptic ulcer, gastrointestinal complications, and inhibition of prostaglandin synthesis is directly linked to the

continued intake of ACF. Biotransformation of ACF into diclofenac leads to selective inhibition of COX-2. Furthermore, some previous research on NSAIDs has discovered that these drugs can cause liver toxicity and other side effects; however, the extent of these side effects is uncertain [6].

Silymarin is an extract of *Silybum marianum* (milk thistle) containing polyphenolic flavonoid compound, silybin being the most important one. It acts as a radical scavenger and therapeutic agent by reducing free radical production and ameliorating damage caused by xenobiotics. Silymarin is used to treat liver injuries and severe hepatitis [7]. Studies have shown that silymarin shows protective aptitude towards hepatic, renal and gastric damage [8]. Silymarin has ability to stabilize cell membranes and enhance protein production [9]. *In vivo* investigations have shown a remarkable hepatoprotective effect of silymarin on liver injuries of diverse etiology [10].

In normal body conditions, endogenous antioxidants are involved in controlling ROS production. The main reason of oxidative stress is loss of proportion between ROS production and antioxidants; thus, this oxidative stress interferes with a lot of cellular functions and leads to different pathological conditions such as inflammation, asthma, aging, and carcinogenesis [11]. Vitamins also known as antioxidants, such as vitamin C, are also important in regulating metabolic signaling pathways that lead to the organs' perfect activities. Synthetic medicines for treating liver injuries can be less efficient and have disastrous effects. Different natural plants and microbes are used as the main component in the manufacturing process of medicinal products. Plants naturally contain different nutrients and antioxidants that integrate into their defense system to protect the plant itself and other organisms from damage induced by reactive substances. The world has recognized the importance of plant-based antioxidants, i.e., phenols and flavonoids as a treatment for tissue ailments and injuries [12-14].

Telosma cordata (Burm. f.) Merr, locally known as Chinese Violet, is a perennial creeper and belongs to the family Apocynaceae, a native plant of South China, India, Burma, and Indochina [15]. It is generally cultivated in different parts of Southeast Asia, especially in Thailand, Malaysia, and Vietnam. *T. cordata* is used as an antidote, tranquilizer, and antipyretic agent. *In vitro* cytotoxicity of methanolic extract of leaves of *T. cordata* was found against Hep-G2 (haptonema carcinoma). It is found to be effective against backache and hematuria [17,18]. In this study, we evaluated the antioxidant and anti-inflammatory properties of *T. cordata* against Aceclofenac-induced hepatotoxicity in rats.

Methodology

Plant material

Fresh leaves of *T. cordata* were collected from the area near the Faisal Mosque of Islamabad, Pakistan in September 2018 and was identified by Dr. Syed Afzal Shah. A voucher specimen (#130430) was deposited at the Pakistan Herbarium, Quaid-i-Azam, University, Islamabad.

Extract preparation: Leaves were air dried under shade in a non-humid area for almost 22 days, and then the dried plant was converted to a fine powder (1.3 kg) by a 60-mesh size willy-mill. Ninety five percent methanol was used at a ratio of 1:3 to soak plant powder and reflexed for 72 h (thrice). Filtration was carried out to remove solid material and desired fractions *viz* n-Hexane (TCH), Chloroform (TCC), Ethyl acetate (TCE), and n-Butanol (TCB), Aqueous (TCA) were prepared according to the standard protocol of Toma et al. [19]. All the fractions were dried and kept at -4°C for further investigations.

In vitro studies

Qualitative phytochemical screening: The identification of phytochemical constitutes such as alkaloids, phenols, flavonoids, cardiac glycosides, coumarins, saponin, tannin, anthraquinone, flavonoid, and triterpenes was assessed in various prepared fractions of *T. cordata* by using standard procedures described by Saima et al. [20]. Plant extracts were boiled with distilled water and relevant solutions were prepared for qualitative phytochemical analysis.

Quantitative phytochemical evaluation: Total tannin, phenolic, and flavonoid content of various extracts of *T. cordata* were evaluated by standard methods of Van Buren and Robinson, Danilewicz, and Park et al., respectively [21-23]. The calibration curve of rutin ($y=0.0013x + 0.0914$: $R^2=0.9914$) for flavonoid while gallic acid curve ($y=0.0016x + 0.1459$, $R^2=0.9815$) for phenol and tannin content was constructed and values were represented as milligrams of RE/g and GAE/g respectively.

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay: The protocol designed by Chan et al. was used to calculate the radical scavenging ability of plant extracts against free radicals produced by DPPH [24]. 500 µl of various concentrations (31.25 µg/ml to 1000 µg/ml) of plant fractions and crude extract were mixed with 1 ml of DPPH solution (0.1 mM). DPPH was added to 500 µl of methanol and taken as a control. Experiment was performed in triplicates and the following formula was applied to find out the percentage scavenging activity of extracts against DPPH:

Scavenging activity = {1-Absorbance of the sample}/Absorbance of control × 100

Bovine Serum Albumin (BSA) assay: Assay protocol of the BSA test described by Williams et al. was used with some amendments to ascertain the anti-inflammatory properties of the plant fractions [25]. BSA (5%) was dissolved in Tris Buffered Saline to prepare the BSA solution. Different concentrations of plant extracts (300 µl) and positive control (aspirin) were mixed with 1000 µl of BSA solution. A mixture of BSA solution and buffer was taken as a control. Protein denaturation was initiated by heating the solution at 70°C for 15 min. The solution was given incubation for 10 min at ambient temperature. The activity of the solutions (level of protein precipitation) was measured at 660 nm. The experiments were conducted in triplicate and the percentage of inhibition of protein denaturation was evaluated by using the following equation:

*% Anti-Denaturation Activity = (Absorbance of control – Absorbance of sample)/Absorbance of control × 100

High-Performance Liquid Chromatography (HPLC): Flavonoids and phenolics analysis of TCM extract by HPLC method was carried out using an Agilent 1200 series, Germany, particle size; 5 µm and capacity for separation is 25 ml, equipped with UV detector. The sample was dissolved with chromatographic-grade methanol. The test solution and standard were passed through 0.45 µm membrane filter paper for filtration. Separation of polyphenols in each sample was performed using a column Zorbex plus RSC8 (Agilent U.S.A), reverse phase analytical at 25°C. Mobile phases A: Acetonitrile (5%); methanol (10%); water (85%); acetic acid (1%) and phase B: methanol (60%); acetonitrile (40%); acetic acid (1%). The applied flow rate and injection volume was kept as 20 µl/min and 1.20

ml respectively. Standard peaks were used for the quantification of various compounds at different wavelengths.

In vivo studies

Ethical statement: The ethical committee of Quaid-i-Azam University, Pakistan provided the ethical approval (Bch# 311), and all the investigations were carried out by following the standard US guidelines.

Acute toxicity study: Before starting the experimentation, acute toxicity test was performed by following the guidelines of OECD. Female rats (n=6) weighing 130 g to 150 g body weight (bw) were used for the study and the experiment was started at an initial dose of 50 mg/kg bw of TCM for 14 days. The experiment was further extended on male rats (n=6) for 14 days with different concentrations of TCM (100 mg/kg, 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 3000 mg/kg bw) as no signs of distress, pain, or toxicity were found at the initial stage. All the rats were observed on daily basis and no sign of toxicity was found, hence 150 mg/kg and 300 mg/kg bw doses were chosen to analyze the hepatoprotective potency of TCM [26]. Moreover, blood samples were collected on the last day of the experiment, and hematological profile was studied.

Study design

Methanol extract of *T. cordata* (TCM) was selected for *in vivo* studies. Forty-two Sprague-Dawley male rats with weights ranging between 160 g to 210 g were selected from the Animal Primate Facility of Quaid-i-Azam University. Basal diet and water were provided to rats and temperature was maintained at (25°C ± 2°C) with a 12 h dark/light cycle.

Dose plan: Rats were segregated into seven different groups and supply of feed and water to the rat's cages was halted one day before initiating this experiment.

Group I: received 0.9% saline and labeled as control.

Group II: ACF (60 mg/kg b.w.)

Group III: ACF and Silymarin (200 mg/kg b.w.)

Group IV: ACF and TCM (150 mg/kg b.w.)

Group V: ACF and TCM (300 mg/kg b.w.)

Group VI: TCM (150 mg/kg b.w.) alone

Group VII: TCM (300 mg/kg b.w.) alone

Total 18 doses were administered after every 24 h gap for 36 days and after completion of experiment, rats were dissected. Blood was collected from ventral side of heart and liver was removed, washed with chilled saline, and weighed. One piece of each organ was stored for biochemical analysis and the second piece was preserved in 10% formalin for histopathological studies.

Body and organ weight: Weight of all the rats was observed at the start and then on the last day of the experiment. Initial and final body weights were compared to calculate the percentage increase in body weight of rats. On the time of liver removal from dissected body, relative weight of liver was measured separately.

Biochemical assessment of serum: Serum markers such as Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), total bilirubin and albumin were analyzed by AMP Diagnostic kits (Krenngasse 8010 Graz, Austria) after collection of serum by centrifugation of blood at a specific speed of 3000 ×g for 20 min.

Preparation of homogenate: For the preparation of liver homogenate, 100 mg of liver tissue was taken and homogenized in already prepared 1 ml of K_2PO_4 buffer with a molarity of 100 mM and containing EDTA with a molarity of 1 mM (pH=7.4). Prepared homogenate was then centrifuged at 1500 ×g at a maintained temp of 4°C for half an hour. After centrifugation, pellet was discarded, and the supernatant was collected for enzymatic assays.

Protein estimation: The method of Lowry and Rosebrough [27] was followed for the estimation of total soluble protein in liver homogenate. BSA was used as a standard to measure the protein contents.

Antioxidant enzyme evaluation: Standard protocols of Chance and Maehly, Kakkar et al., and Jollow et al. were followed to evaluate the activity of *in vivo* antioxidant enzymes including Catalase (CAT), Superoxide Dismutase (SOD), Peroxidase (POD), and reduced Glutathione (GSH), respectively [28-30]. Estimation of enzyme activity was done by recording absorbance change of reaction mixture as 0.01/min of activity and presented as Unit/mg of protein for CAT, SOD, and POD assay and µM GSH of protein for glutathione assay.

Estimation of lipid peroxidation and hydrogen peroxide (H_2O_2): Estimation of levels of Thiobarbituric Acid Reactive Species (TBARS) was done by adding 0.1 M phosphate buffer, homogenate, 100 mM ascorbate, and 100 mM ferric chloride solution to test tube [31]. Absorbance change of reaction mixture was noted at 535 nm. Activity was designated as nM TBARS/min/mg tissue. Levels of Hydrogen Peroxide (H_2O_2) were determined by the ability of horse reddish peroxidase to oxidize phenol red by H_2O_2 [32]. Calculation was done by comparing absorbance change of reaction mixture with blank at 610 nm and expressed as H_2O_2/min/mg tissue.

Nitrite assay: To estimate the level of nitrite, Griess reagent dependent Grisham et al. methodology was applied [33]. Homogenate was dissolved in 3.0 M NaOH and 5% $ZnSO_4$ solution. After centrifugation at 6400 ×g, supernatant was dissolved in Griess reagent. Absorbance was observed at 540 nm whereas calibration curve was drawn against sodium nitrite to define the concentration of nitrites.

Histopathological examination of tissues: For histopathological investigation, liver tissues were preserved in 10% formalin. Washing of those tissues was done in increasing concentration of alcohol i.e., 50%, 70%, 90% and 100%. 4 um to 5 um thin tissue sections were embedded in paraffin and hematoxylin/eosin dye was used for staining the tissues. Light microscope (DIALUX 20 EB) was used to examine the slides at 40x magnification.

Statistical analysis

Results are represented as means ± standard deviation. For the determination of significance among variables, one-way ANOVA was performed by using statistics 8.1 software. A p-value of ≤ 0.05 was assumed to be significant in Tukey HSD Post hoc comparison. Results are analyzed and presented using Graph prism 5.0 and Microsoft excel 2010.

Results

Yield of fractions of *Telosma cordata*

The extraction yield of methanolic extract of plant was approximately 55 g. Upon fractionation 8.1 g of Hexane Fraction

Table 1: Qualitative analysis of *T. cordata*.

Phytochemicals	TCM	TCH	TCC	TCE	TCB	TCA
Alkaloids	+	-	-	-	-	+
Coumarins	+	-	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	-
Phenols	+	+	+	+	+	+
Saponins	+	+	+	-	+	+
Anthraquinone	+	+	+	-	-	-

Table 2: Quantitative analysis of extracts of *Telosma cordata*.

	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/g)	Total tannin content (mg GAE/g)
TCM	296.10 ± 3.14^{b}	44.82 ± 0.88^{b}	309.56 ± 1.27^{a}
TCH	65.89 ± 2.19^{e}	20.40 ± 0.90^{f}	74.11 ± 2.09^{d}
TCEA	193.81 ± 3.90^{c}	28.15 ± 2.31^{e}	115.64 ± 1.46^{c}
TCC	334.64 ± 1.91^{a}	57.89 ± 1.77^{a}	301.61 ± 1.68^{a}
TCB	170.89 ± 1.80^{d}	38.92 ± 2.03^{c}	254.11 ± 2.41^{b}
TCA	289.22 ± 3.61^{b}	32.71 ± 1.17^{d}	30.77 ± 0.24^{e}

(Mean ± SD); Mean with different superscripts specify significance at p <0.05
TCM: *Telosma cordata* Methanol Extract: TCH: *Telosma cordata* Hexane Fraction: TCEA: *Telosma cordata* Ethyl Acetate Fraction; TCC: *Telosma cordata* Chloroform Fraction; TCB: *Telosma cordata* Butanol Fraction; TCA: *Telosma cordata* Aqueous Fraction

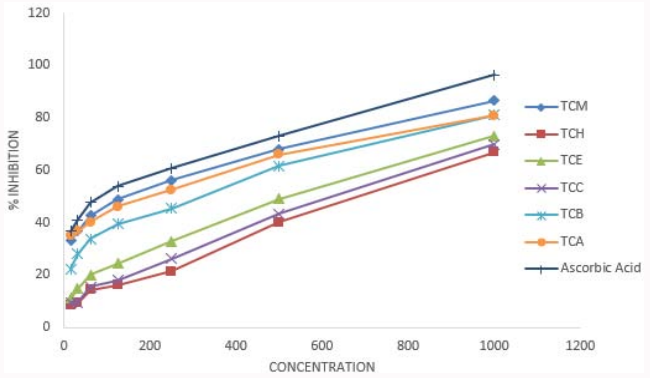

Figure 1: % Inhibition of DPPH free radicals versus various concentrations of *Telosma cordata* fractions.
TCM: *Telosma Cordata* Methanol Extracts; TCH: *Telosma Cordata* Hexane; TCE: *Telosma Cordata* Ethyl Acetate; TCC: *Telosma Cordata* Chloroform; TCB: *Telosma Cordata* Butanol; TCA: *Telosma Cordata* Aqueous Fractions

(TCH), 4.3 g Chloroform (TCC), 6.2 g Ethyl Acetate (TCE), 8.5 g Butanol (TCB) and 19 g of aqueous fraction (TCA) were obtained.

Phytochemical analysis/study: A number of qualitative assays were performed to identify many different types of phytochemicals naturally present in prepared fractions of *T. cordata*. Results of these studies are illustrated in Table 1. Tannins, phenols, and coumarins were found in each fraction of the plant sample whereas alkaloids were found only in aqueous fraction (TCA). Presence of saponins in each fraction except TCE was ensured by these assays. Flavonoids were majorly observed in two fractions including TCM and TCB but absent in all other fractions. TCA, TCB and TCE fractions did not contain anthraquinone though other three fractions contained these phytochemical contents.

Quantitative identification

The quantitative phytochemical analysis of *T. cordata* indicated

Table 3: Antioxidant and anti-inflammatory activity of *T. cordata*.

	DPPH assay IC_{50} (µg/ml)	BSA assay IC_{50} (µg/ml)
TCM	212.2 ± 3.6^{f}	229.7 ± 3.1^{f}
TCH	702.6 ± 4.2^{a}	797.6 ± 4.3^{a}
TCE	576.3 ± 2.8^{c}	543.3 ± 2.4^{c}
TCC	649.8 ± 3.4^{b}	718.8 ± 4.6^{b}
TCB	380.3 ± 2.9^{d}	489.9 ± 2.6^{d}
TCA	261.3 ± 3.1^{e}	310.6 ± 1.8^{e}
Ascorbic acid	131.7 ± 3.9^{g}	
Aspirin		61.48 ± 1.1g

(Mean ± SD); Different superscripts specify significance at p <0.05
BSA: Bovine Serum Albumin

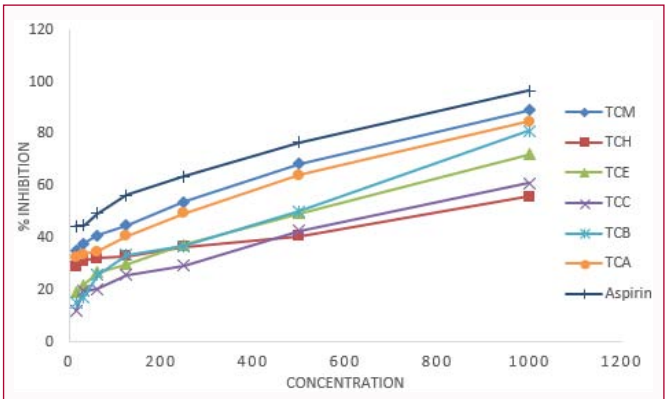

Figure 2: % Inhibition of albumin denaturation *versus* various concentrations of *Telosma cordata* fractions.

that TCC and TCM have a high amount of flavonoid, phenol, and tannin content (57.89 ± 1.77 and 44.82 ± 0.88 mg RE/g), (334.64 ± 1.91 and 296.10 ± 3.14 mg GAE/g), and (301.61 ± 1.68 and 309.56 ± 1.27 mg GAE/g) respectively. At the same time, TCH exhibited the lowest amount of phenolics, flavonoids, and tannins. The quantitative percent yield of phytochemicals for various extracts of *T. cordata* are shown in Table 2.

Free radical scavenging potential of *T. cordata*

The crude extract and fractions of *T. cordata* converted the stable, purple-colored DPPH into diphenyl hydrazine (yellow) due to its scavenging power against DPPH. Figure 1 represents the % inhibition of free radicals by *T. cordata* fractions. Among all the extracts, methanol extract of plant exhibited the highest % inhibition of free radical (highest scavenging activity) at all doses; however, TCM was considerably ($p<0.05$) less effective than the scavenging activity of ascorbic acid. Table 3 shows that Methanol and Aqueous (TCM and TCA) fractions have the lowest IC_{50} values, while then-hexane fraction had the highest IC_{50} value compared to ascorbic acid.

Inhibition of protein denaturation

Figure 2 shows % inhibition of protein denaturation as aspirin and methanol extract exhibited the highest (96.61% and 89.07%, respectively) activity at a concentration of 1000 µg/ml. Table 3 represents that methanol extract had the lowest IC_{50} value (229.7 ± 3.1 µg/ml) among various extracts, while aspirin had an IC_{50} value of 61.48 ± 1.1 µg/ml.

HPLC-DAD analysis

HPLC analysis indicated the presence of vanillic acid (26.11 ± 0.21 µg/mg), rutin (18.08 ± 0.14 µg/mg), Gallic acid (25.2 ± 0.30 µg/

Table 4: Treatment effects of *Telosma cordata* on hematological profile.

	WBC (10^3/mm^3)	RBC (10^6/mm^3)	Neutrophil (10^3/mm^3)	Platelets (10^3/ml)	LYM (10^3/mm^3)	MCHC (g/dl)	Hemoglobin (g/dl)
Control	7.31 ± 0.21^c	6.69 ± 0.29^a	24.04 ± 0.37^c	172.2 ± 2.05^c	4.01 ± 0.13^a	6.91 ± 0.35^b	12.04 ± 0.13^c
TCM (1500 mg/kg bw)	10.6 ± 0.11^a	6.82 ± 0.27^a	27.-2 ± 0.24^a	204.5 ± 2.81^b	4.31 ± 0.11^a	6.89 ± 0.20^b	15.02 ± 0.19^b
TCM (3000 mg/kg bw)	8.82 ± 0.22^b	7.21 ± 0.14^a	30.12 ± 0.17^b	238.5 ± 2.01^a	5.31 ± 0.23^b	10.01 ± 0.91^a	16.72 ± 0.36^a

Mean ± SD (n=6 female rats). Different superscripts show significance at p<0.05
TCM: *Telosma cordata* Methanol Extract; WBC: White Blood Cells: RBC: Red Blood Cells; LYM: Lymphocytes; MCHC: Mean Corpuscular Hemoglobin Concentration

Table 5: Effect of *Telosma cordata* on body weight and organ weight of rats.

Treated Groups	Initial Body Weight (g)	Final Body Weight (g)	% Increase in body weight	Absolute Liver weight (g)	Relative Liver Weight (g)
Control	125 ± 1.7	190 ± 2.6	52.3 ± 1.3^a	6.4 ± 0.3^f	35.7 ± 1.6d^e
ACF (60 mg/kg)	127 ± 2.6	158 ± 2.3	24.5 ± 1.08^e	8.9 ± 0.8^a	56.3 ± 1.9^a
ACF+Silymarin (200 mg/kg)	126 ± 2.7	187 ± 2.48	48.4 ± 2.2bc	7.1 ± 0.4^d	37.9 ± 1.5^d
ACF+TCM (150 mg/kg)	125 ± 2.5	166 ± 2.1	32.4 ± 1.3^d	8.1 ± 0.7^b	48.7 ± 1.5^b
ACF+TCM (300 mg/kg)	128 ± 2.6	187 ± 1.7	46.1 ± 2,0^c	7.5 ± 0.5^c	40.1 ± 1.6^c
TCM (150 mg/kg)	127 ± 1.6	189 ± 1.4	48.8 ± 2.4^b	6.7 ± 0.4^e	35.0 ± 1.2^e
TCM (300 mg/kg)	129 ± 1.16	192 ± 1.8	48.8 ± 1.9^b	7.0 ± 0.4^d	36.0 ± 0.9^{de}

Mean ± SD (n=6), Different superscript letters specify significance at p<0.05
TCM: *Telosma cordata* Methanol Extract; ACF: Aceclofenac

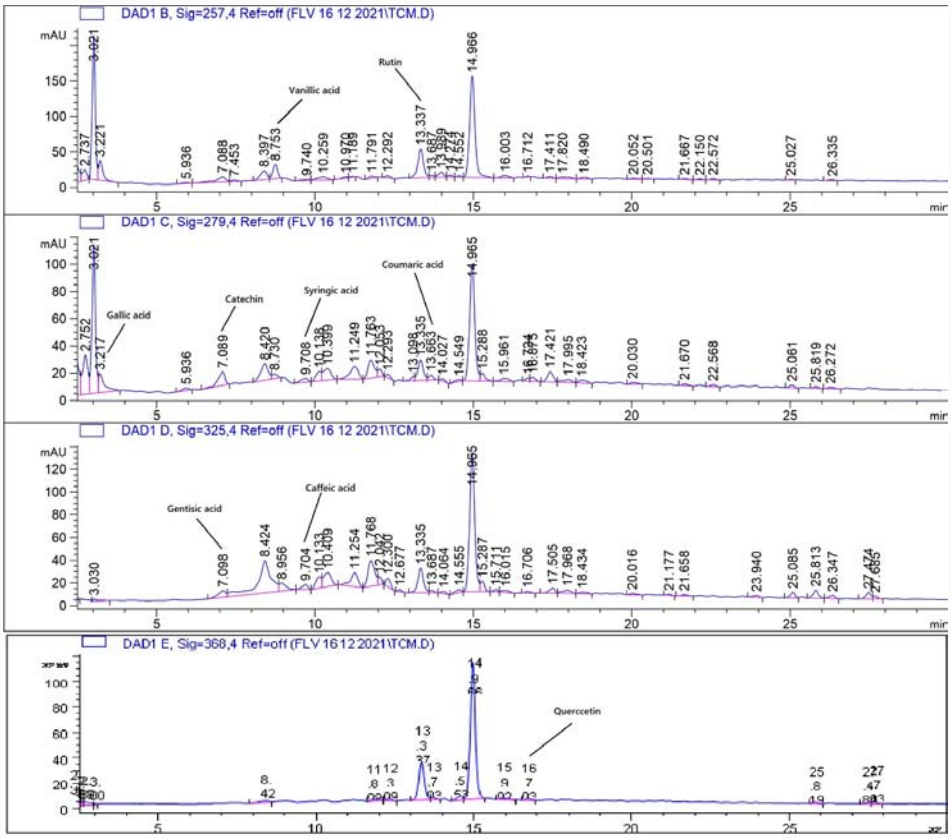

Figure 3: HPLC-DAD analysis of *Telosma Cordata* Methanol Extract (TCM).

mg), catechin (20.5 ± 0.23 µg/mg), syringic acid (16.5 ± 0.26 µg/mg), coumaric acid (4.77 ± 0.05 µg/mg), gentisic acid (16.8 ± 0.08 µg/mg), caffeic acid (3.81 ± 0.16 µg/mg), and quercetin (6.38 ± 0.07 µg/mg) in TCM. HPLC chromatograms of TCM are presented in Figure 3.

Hematological profile of acute toxicity

The hematological profile of rats was studied as rats showed no toxicity and normal behavior pattern during experimentation. Anyhow, the analysis showed that there was a non-considerable change (p>0.05) in Red Blood Cells (RBCs) at different doses. In contrast, lymphocytes showed significant elevation (p<0.05) at 3000 mg/kg bw of TCM. However, a significant (p<0.05) change was found in the levels of white blood cells, neutrophils, and hemoglobin as presented in Table 4.

T. cordata effects on body weight and liver weight

ACF-induced toxicity reduced the percent increase in body weight while a considerable (p<0.05) increase was found in relative

Table 6: Effect of *Telosma cordata* on serum markers.

	AST (U/l)	ALT (U/l)	ALP (U/l)	Albumin (mg/dl)	Bilirubin (mg/dl)
Control	72.3 ± 1.3^{g}	68.9 ± 1.5^{g}	74.1 ± 2.4^{g}	5.24 ± 0.18^{a}	0.74 ± 0.04^{de}
ACF (60 mg/kg)	137.5 ± 3.4^{a}	103 ± 2.9^{a}	156.9 ± 3.8^{a}	2.94 ± .14^{g}	1.51 ± 0.02^{a}
ACF+Silymarin (200 mg/kg)	92.5 ± 2.5^{d}	55.8 ± 1.7^{f}	91.5 ± 2.4^{d}	4.85 ± 0.11^{d}	0.95 ± 0.07^{ef}
ACF+TCM (150 mg/kg)	120 ± 2.7^{b}	91.4 ± 2.7^{b}	123.6 ± 3.4^{b}	3.90 ± 0.07^{f}	1.02 ± 0.06^{b}
ACF+TCM (300 mg/kg)	102 ± 2.6^{c}	83.4 ± 3.0^{c}	108.9 ± 3.5^{c}	4.42 ± 0.15^{e}	0.93 ± 0.07^{c}
TCM (150 mg/kg)	80.7 ± 2.5^{e}	71.1 ± 2.8^{d}	80.9 ± 2.3^{e}	5.05 ± 0.17^{c}	0.84 ± 0.06^{d}
TCM (300 mg/kg)	74.2 ± 2.5^{f}	69.7 ± 2.4^{e}	72.9 ± 2.5^{f}	5.26 ± 0.27^{b}	0.72 ± 0.03^{e}

Mean ± SD (n=6), Different superscripts specify significance at p<0.05
TCM: *Telosma cordata* Methanol Extract; ACF: Aceclofenac

Table 7: Effect of *Telosma cordata* on biochemical markers.

	H_2O_2 (nM/min/mg tissue)	Nitrite (µM/mg protein)	TBARS (nM/min/m protein	Protein (µg/mg/tissue)
Control	2.08 ± 0.5^{d}	58.6 ± 1.1^{e}	43.8 ± 0.7^{f}	15.6 ± 0.3^{a}
ACF (60 mg/kg)	11.5 ± 3.2^{a}	133 ± 1.5^{a}	74.6 ± 3.5^{a}	10.2 ± 0.3^{f}
ACF+Silymarin (200 mg/kg)	2.95 ± 1.0^{c}	66.3 ± 0.5^{d}	53.7 ± 0.8^{d}	15.0 ± 0.03^{b}
ACF+TCM (150 mg/kg)	6.86 ± 2.1^{b}	116 ± 2.0^{b}	62.3 ± 2.1^{bb}	11.5 ± 0.03^{e}
ACF+TCM (300 mg/kg)	2.84 ± 1.1^{cd}	78.4 ± 0.8^{c}	58.4 ± 0.7^{c}	12.2 ± 0.09^{d}
TCM (150 mg/kg)	2.01 ± 1.0^{e}	60.0 ± 0.7^{d}	47.2 ± 1.03^{e}	14.1 ± 0.1^{c}
TCM (300 mg/kg)	2.09 ± 1.4^{d}	57.4 ± 0.2^{e}	46.7 ± 1.04^{e}	15.7 ± 0.03^{a}

Mean ± SD (n=6), Different superscripts specify significance at p<0.05
TCM; *Telosma cordata* Methanol Fraction; ACF: Aceclofenac

and absolute liver weights of rats. In contrast, silymarin treatment showed ameliorating effects against ACF-induced toxicity as body and organ weights of this group were similar to normal rats. Co-administration of TCM (150 mg/kg bw and 300 mg/kg bw) with ACF suppressed the toxic effects of ACF and showed an increase in body weight whereas restoration of absolute and relative liver weight was also observed. Administration of rats with only plant dose (150 mg/kg bw and 300 mg/kg bw) depicted a significant (p<0.05) elevation in body weight (Table 5).

T. cordata restored serum markers in liver

After administration of ACF, an increase in levels of specific liver serum enzymes such as ALP, AST, ALT, and total bilirubin was observed (Table 6). Though, albumin showed a significant decrease in its level. Increased levels of various serum enzymes are associated with hepatic damage with loss of integrity of cells. By contrast, co-administration of ACF with silymarin significantly (p<0.05) restored the level of these serum enzymes towards the control rats. Likewise, simultaneous treatment of Aceclofenac and TCM doses (150 mg/kg bw and 300 mg/kg bw) showed a remarkable effect (p<0.05) in repressing the levels of serum markers, yet an increase in albumin level was also notable.

T. cordata restored antioxidant enzymes in liver

Figure 4 shows levels of antioxidant enzymes in liver tissue after TCM treatments. Upon ACF administration, tissue damage was observed with a significant (p<0.05) reduction in the level of catalase, superoxide dismutase, peroxidase, and reduced glutathione. Although treatment with silymarin along with ACF showed a protective effect against liver injury, as ensured by higher levels of these enzymes. Co-administration of TCM doses with ACF reportedly (p<0.05) elevated the levels of these enzymes in comparison to the rats treated with ACF only. However, TCM alone showed no effect on the biological activities of these enzymes.

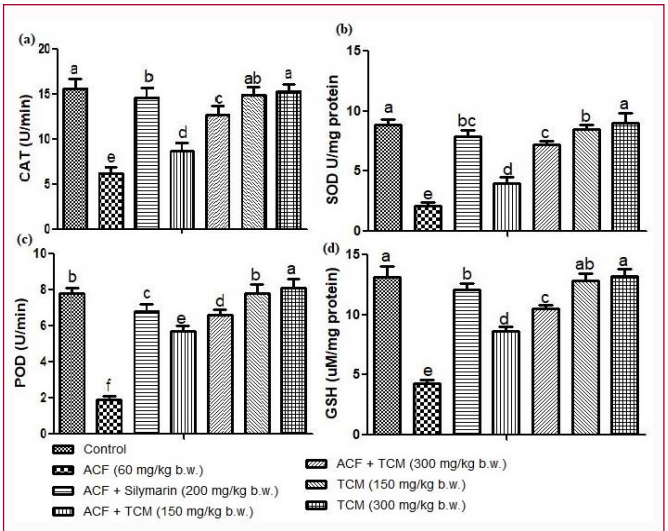

Figure 4: Effect of TCM on antioxidant enzymes of the liver.
CAT: Catalase; SOD: Superoxide Dismutase; POD: Peroxidase; GSH: Reduced Glutathione

T. cordata reduced toxicity in liver tissues

Table 7 summarizes the hepatoprotective ability of TCM on levels of H_2O_2, nitrite, TBARS, and total protein. Total protein was decreased, while a significant (p<0.05) increase in H_2O_2, nitrite, and TBARS was noticed in ACF-treated rats compared to normal rats. TCM administration with ACF markedly (p<0.05) reduced the concentration of biochemical markers while an elevation was observed in protein level. Rats treated with TCM alone did not affect the levels of biochemical markers. By reducing the hepatotoxicity caused by ACF, silymarin administration showed a significant (p<0.05) restoration of nitrites, TBARS, H_2O_2, and total protein toward the normal level.

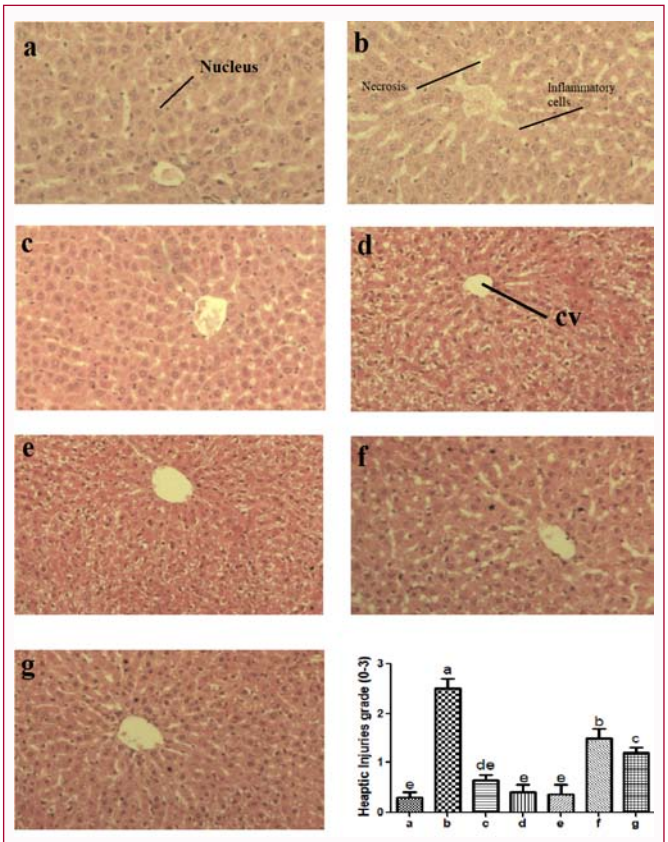

Figure 5: Histopathological analysis of liver tissues after different treatments (CV: Central Vein).

Protective role of *T. cordata* on histology of liver

Liver of normal rats showed a normal architecture with intact hepatocytes, central vein and equally distributed cytoplasm (Figure 5A). Liver of ACF treated rats showed severe injuries such as sinusoidal obstructions, necrosis, fibrosis, and hepatocellular degeneration as alterations were prominent in morphology of tissue (Figure 5B). Administration of high dose of TCM with ACF showed improvement in morphological structure of liver by reducing the liver damages and injuries hence showing protection against liver toxicity while rats administered with low dose of plant TCM along with ACF showed necrosis and some injuries. Liver of rats from TCM alone treated groups had shown normal morphological features of liver (Figures 5D-5G). Silymarin (200 mg/kg bw), a standard drug, was given to restore structural abnormalities and cellular anomalies as shown in Figure 3 and 5C.

Discussion

Toxic chemical substances cause liver injuries in body which induces oxidative stress and inflammation. Natural antioxidants and food additives help to reduce induced stress and toxic effects of xenobiotics by scavenging free radicals. Humans have been using plants as a source of conventional medicines for hundreds of years. Many herbal extracts, prepared from plants, have been examined for their antioxidant, anti-inflammatory, and hepatoprotective properties against liver injuries in various experimental animal models [34]. In the present study, a phytochemical qualitative study confirmed the presence of alkaloids, flavonoids, tannins, coumarins, phenols, and saponins in *T. cordata* fractions.

The production of ROS is correlated with a number of severe and persistent body disorders which embraces liver, pulmonary, neurodegenerative, and digestive infections. Diet is a rich source of antioxidants and can help the body to regulate the deficiency of antioxidants endogenously and thus restore all the damage caused because of oxidative stress.

Phenolics and flavonoids such as rutin, quercetin, gallic acid, catechin and caffeic acid were found in the HPLC analysis of TCM. Rutin is a well-known standard antioxidant and protective compound against toxicity and getting recognition due to its medicinal properties [35]. On the other hand, catechin shows strong activity against free radicals thus having antioxidant activities [36]. Thus, the presence of these compounds in TCM extract could be the reason for scavenging free radicals when evaluated against DPPH and showing antioxidant activity. The presence of antioxidants in TCM is also linked with the inhibition of denaturation of bovine serum albumin and showing anti-inflammatory potential. Our findings are similar to the studies of Naz et al. which claimed that phytochemicals of *Pilea umbrosa* have antioxidant properties [34].

ACF has been identified as commonly associated with hepatotoxicity and oxidative stress and it is also assumed as the major cause of cellular disorder by damaging the antioxidant enzymatic system, acute hepatitis, and increasing peroxidation of lipids. Hepatotoxicity from NSAIDs can be found at any time after initiation of treatment, but like most severe drug reactions, it occurs after 6 to 12 weeks after administration. In the liver, ACF causes activate lipid peroxidation, necrotic and apoptotic cell death, and deterioration of membranal structure leading to the secretion of enzymatic components in blood. According to previous research, a significant increase in levels of biochemical markers i.e., ALP, ALT, and AST can be seen after oral induction of animal models with Aceclofenac [5,37]. To access the protective potential of our plant, an Aceclofenac exposed system is created in rats. Our research has clearly demonstrated that treating the animal models with Aceclofenac at higher dose for about eight weeks on alternate days resulted in the induction of hepatic damage and oxidative stress, while *T. cordata* showed promising protective results against stress and organ damage. Our results are in line with a previous study [37].

This study has also found that TCM did not cause injurious alterations in hematological markers, including the count of HB, MCV, WBC, HCT, RBC, and platelets in rats, even at a high dose of 3000 mg/kg. TCM is found to be non-toxic, as any signs of discomfort or pain was not obvious in TCM-treated rats. Similar results for the methanolic extract of the plant are declared by previous findings [34].

Body weight is an important factor in the conflicting effects of hepatotoxins and is replicated as an element that limits toxicity studies. In the current study, the percent increase in body weight of rats was reduced, whereas absolute and relative liver weight was enhanced with ACF administration. Basically, NSAIDs induce oxidative stress by producing free radicals in the body that cause an increase in the organ weight by affecting the metabolic process of different organs. Same results are represented by previous research Bort et al. [38].

Naturally, our body has a specific antioxidant defense mechanism that includes endogenous enzymatic antioxidants such as CAT, SOD, POD, and glutathione. These enzymes along with exogenous antioxidants protect the organ and body from damage caused by oxidative stress by capturing ROS. Catalase has ability to neutralize hydrogen peroxide H_2O_2 *via* a catalytic transformation or through

peroxidation metabolism [39] while superoxide dismutase is a metallic enzyme that helps in the conversion of superoxide radicals to H_2O_2 and O_2 by dismutase reaction to reduce the toxicity [40]. Glutathione peroxidase causes the neutralization of peroxides and thus providing protection against toxicity of ROS. Abnormal necrosis in liver is stimulated by decreased levels of GSH [41]. All these antioxidant enzymes and GSH level in cells were reduced markedly in ACF treated group, which have caused injury in liver cells through deposition of ROS. Administration of TCM along with ACF has significantly restored antioxidant enzyme concentration near to normal range. TCM contains many bioactive components which are efficient in balancing the levels of antioxidants as well as in combating different infectious diseases. Increased lipid peroxidation is associated with higher levels of TBARS that result in tissue destruction and failure of antioxidant defense mechanisms [5,35]. ACF-intoxicated rats treated with TCM (300 mg/kg) reduced the lipid peroxide levels compared with rats treated with ACF only.

An extreme notch of disruptions in hepatic cells consisting of cellular hypertrophy, intrusion of inflammatory cells, intrusion of the central lobule, and sinusoidal obstruction was observed in rats treated with ACF dose through histological examination of tissues. Low/high doses of TCM with ACF dose reduced these losses in the body tissues. No morphological changes were observed in the liver anatomy of groups treated with TCM doses alone. Similar results have been declared by Gupta and Pandey, who claimed that *Terminalia bellirica* fruit extract has a protective aptitude against Aceclofenac endured hepatic damage [6]. Many biocomponents and polyphenols are present in TCM extract, which have potential antioxidant properties and thus help the plant to prevent tissue damage by reducing peroxidation of lipids in body organs, including the liver.

Conclusion

The current study illustrated the potent antioxidant and anti-inflammatory propensity of *T. cordata* extract. The presence of flavonoids and polyphenols are claimed for the possible antioxidant, anti-inflammatory and hepatoprotective mechanism of *T. cordata*. Our data also revealed protective effect of TCM by regulating the levels of enzymatic markers, anti-inflammatory mediators and serum markers against the intoxicated effects of ACF. Moreover, TCM reduces the injuries of liver architecture, hence restoring the hepatic morphology.

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