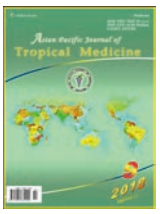

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Ameliorative effect of *Pergularia daemia* (Forssk.) Chiov. leaves extract against anti-tuberculosis drugs induced liver injury in rats

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ABSTRACT

Objective: To evaluate therapeutic potential of hydroethanolic extract of *Pergularia daemia* (*P. daemia*) against anti-tuberculosis drugs (ATDs) induced liver injury. **Methods:** Wistar albino rats were divided into seven groups of six animal in each. The ATDs and *P. daemia* extract (100, 200 and 400 mg/kg, *p.o.*) were conjointly administered for 8 weeks and various biochemical, histoarchitectural, ultrastructural studies were performed. **Results:** Administration of ATDs significantly increased aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, triglycerides, cholesterol, bilirubin and decreased glucose and albumin level. Increased lipid peroxidation and reduction in glutathione, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase were found after ATDs exposure. Administration of *P. daemia* extract maintained serum biochemical indices as well as antioxidant status similar to control and diminished oxidative stress in dose dependent manner. Histological and ultra-structural observations substantiated biochemical findings. **Conclusions:** *P. daemia* has therapeutic potential against ATDs induced liver injury and may be of clinical significance after extensive studies.

1. Introduction

Tuberculosis (TB) is one of the leading causes of death. There were about 10.4 millions new TB cases in 2016[1]. Being major site for drug biotransformation, liver is the most vulnerable organ to chemicals and drugs induced damage. Isoniazid (INH), rifampicin (RIF), ethambutol (ETH) and pyrazinamide (PZA) are antituberculosis drugs (ATDs), which induce injury[2]. Major adverse effects due to these medications are hepatotoxicity[3], hyperuricemia, peripheral neuropathy, rashes, gastrointestinal upset and visual disturbances[4,5]. Adverse effects of drugs may result in discontinuation of TB treatment, which may lead to recurrence or development of drug resistance[6-8].

The *Ayurveda* is natural and holistic medicinal system originated in India, prescribes treatment regimen in many parts of the world. *Ayurveda* mostly relies on plant based drugs and phytochemicals have been the basis for development of many therapeutic molecules in modern medicine[9]. Herbs are easily accessible to human beings in their surroundings and must be investigated for their maximum medicinal potential[10]. Members of the Asclepiadaceae family

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are rich sources of bioactive compounds and they contain a range of different glycosidic substances[11]. *Pergularia daemia* (*P. daemia*) (Forssk.) Chiov. belongs to Asclepiadaceae family, which is used as folk medicine to treat various diseases including liver disorders[12], diabetes[13] and fungal infection[14]. Aqueous and ethanolic root extracts of *P. daemia* possess antioxidant activity due to the presence of flavonoids, alkaloid, steroid, saponins, tannin, glycoside, terpenoids and various phenolic compounds. Bioactive compounds of *P. daemia* such as quercetin, β-sitosterol, β-amyrin, betaine, isorhamnetin, chrysoeriol, taxifolin, naringenin *etc.* are responsible for its wonderful therapeutic potential and free radical scavenging activity[15,16]. It was hypothesized that due to the presence of several biological activities and bioactive phytochemicals, *P. daemia* may have therapeutic potential against RIF, INH, ETH and PZA induced hepatic damage. Thus, present study was planned to explore curative effect of *P. daemia* leaves extract against ATDs induced hepatic injury. The oxidative stress markers, enzymatic and non-enzymatic antioxidant status, tissue biochemistry, optical and ultrastructural observations were taken into consideration to validate therapeutic potential of *P. daemia* extract.

2. Materials and methods

2.1. Chemicals and animals

Pure and analytical grade chemicals, procured from standard chemical dealers were used in the study. Female albino rats (Wistar strain) [(160±10) g] were maintained under standard husbandry condition of light (14 h) and dark (10 h) cycle at (25±2) ℃ temperature and relative humidity of 65%-70%. The rats were fed on standard pelleted diet and water *ad libitum*. Animals were cared for experiments as per the guidelines of institutional animal ethics committee (994/Ere/GO/06/CPCSEA).

2.2. Handling of plants to prepare extract

The whole plant of *P. daemia* was collected from Bilaspur district, Chhattisgarh. The plant identification was made by an eminent botanist and voucher specimen No. GG/C/APO/102 was deposited in the herbarium of Department of Botany, Guru Ghasidas University. Fresh leaves of plants were dried in shade at room temperature, grinded and subsequently sieved to obtain a fine powder. Then 70% ethanolic extract of powder was prepared using Accelerated Solvent Extractor (Dionex ASE, -150). The extract was dried with air blower at room temperature and stored in refrigerator at 4 ℃ till further use.

2.3. No-observed-adverse-effect-level

Aqueous suspension of *P. daemia* extract was prepared in 5% gum acacia and administered to experimental animals at 100, 200, 400 and 800 mg/kg doses for one week. There were no mortality or significant alterations in liver and kidney function markers, No-observed-adverse-effect-level of *P. daemia* extract was found even at the dose of 800 mg/kg in rats (Data communicated elsewhere). Therefore, in the present investigation, aqueous suspension of leaves extract at 100, 200 and 400 mg/kg dose was selected as optimum therapeutic doses. Silymarin (50 mg/kg) in 5% gum acacia was used as positive control.

2.4. Induction of hepatotoxicity by ATDs

First line ATDs, INH (70 mg/kg), RIF (52 mg/kg), PZA (175 mg/kg) and ETH (140 mg/kg) were administered to experimental animals according to instructions given on kit to induce hepatic injury. Doses of ATDs were changed from human doses to the rats using standard formula[17].

2.5. Experimental design

Animals were divided into seven groups of six animals each. Group 1 served as control. Group 2 was treated with *P. daemia per se* at 400 mg/kg, orally. Groups 3–7 were intoxicated with combination of four ATDs (PZA+ETH+INH+RIF) at doses mentioned above for 8 weeks (3 alternative days in a week) and group 3 served as experimental control. Animals of groups 4, 5 and 6 were administered *P. daemia* extract at doses of 100, 200 and 400 mg/kg, *p.o.*, respectively for 8 weeks (3 alternative days in a week considering every next day of ATDs administration respectively). Silymarin (50 mg/kg, *p.o.*) was administered in group 7 as positive control. All the animals were euthanized after 48 h of the last treatment. Blood was collected through puncturing the retro orbital venous sinus just before euthanasia[18].

2.6. Serological biochemical assays

Blood samples were kept at room temperature for 1 h, centrifuged at 3 000 rpm for 10 min and obtained serum was stored at -20 ℃. Serum was used for analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, bilirubin, triglycerides, glucose and cholesterol using diagnostic kits (Erba diagnostics Mannheim GmbH Mallaustr, Germany) according to the manufacturer's instructions.

2.7. Homogenate preparation

Immediately after euthanasia, fresh tissue samples from liver were stored at -20 ℃ for biochemical analyses. For assessment of glutathione (GSH), catalase and superoxide dismutase (SOD) activity, tissues were homogenized in 1% sucrose solution and normal saline solution respectively. The homogenate (10%, w/v) were prepared in hypotonic solution for determination of total proteins,

cholesterol and triglycerides. Homogenate was prepared in 1.15% KCl solution for determination of glutathione metabolizing enzymes *i.e.*, glutathione peroxidase (GPx), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH).

2.8. Microsome preparation for aniline hydroxylase activity

Hepatic tissues were homogenized with tris–HCl buffer (10 mM, pH 7.4) under cold conditions and used for the preparation of microsomes by calcium precipitation method[19]. The activity of aniline hydroxylase was evaluated by recording intensity of colored phenol and *p*-amino phenol (PAP) conjugate at λ_{630} nm and expressed as *n* moles PAP/min/g liver[20].

2.9. Glutathione and TBARS assays

Assay for GSH was performed using dithio nitrobenzoic acid[21] and optical density was noted immediately at λ_{412} nm. The GSH level was calculated using an extinction coefficient of 13 600/M/cm and expressed as μ mole GSH/g tissue. Thio barbituric acid reactive substances (TBARS) were assayed for lipid peroxidation (LPO)[22] in liver tissue and microsomes. LPO was measured in terms of *n* moles TBARS/g tissue using an extinction coefficient of 1.56×10^5/M /cm.

2.10. Assessment of enzymatic antioxidant status

The catalase activity was determined by observing decomposition of H_2O_2 at λ_{240} nm[23]. H_2O_2 concentration was evaluated using extinction coefficient of 0.039 4/mM/cm and catalase activity was expressed in *n* moles H_2O_2/min/ mg protein. Superoxide dismutatse was determined by assessing inhibition of auto oxidation of epinephrine[24]. Specific activity was the percentage inhibition of auto oxidation of epinephrine by the SOD per min and expressed as unit/mg protein.

2.11. Assessment of GSH cycle

Determination of GPx[25], GR[26] and G6PDH[27] were carried out.

2.12. Tissue biochemical assay for cellular biochemistry

Liver tissue was immediately processed for determination of total cholesterol[28], triglycerides[29] and protein[30].

2.13. Histopathological studies

Investigation for histopathology was performed following fixation of tissues in Bouin's fixative and subsequent processing for microtomy at 5 μm thickness. Hematoxylin–Eosin (HE) stained slides were observed under compound light microscope.

2.14. Transmission electron microscopy

Fixation of liver tissues (1 mm^3/pieces) was done in Karnovosky's fixative for 18 h at 4 ℃. Samples were washed with phosphate buffer after fixation. Osmium tetraoxide (1%) was used for Post fixation with 1% osmium tetraoxide followed by dehydration through acetone series. Embedding was done in epon resin and Reichert Jung ultra cut-E microtome was used to cut Ultra-thin sections. The sections grids were stained with uranyl acetate and lead citrate, and observed under transmission electron microscope (TECHNAI).

2.15. Statistical analysis

Results are presented as mean±SE of six animals used in each group. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) at 5% level of significance followed by Student's *t*-test considering $P<0.05$ and $P<0.01$ as significant level[31].

3. Results

3.1. Serological observations

Eight weeks exposure to ATDs elevated liver specific markers ALT, AST, ALP and bilirubin into circulation ($P<0.01$) when compared to control group. All three doses of *P. daemia* (100, 200 and 400 mg/kg) restored deviated liver function markers closer to control significantly ($P<0.01$) in a dose dependent manner (Table 1).

Table 1

Therapeutic effect of *P. daemia* extract against anti-tuberculosis drugs induced toxicity in liver function tests.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)
Group 1	33.90±2.78	72.10±4.06	272.0±19.7	0.17±0.01
Group 2	32.20±2.96	75.20±4.30	261.0±17.8	0.18±0.01
Group 3	72.20±4.57**	110.00±4.71**	458.0±27.8**	0.38±0.02**
Group 4	42.50±3.02$^{\#\#}$	94.60±5.94$^{\#}$	318.0±21.9$^{\#\#}$	0.24±0.02$^{\#\#}$
Group 5	37.10±3.18$^{\#\#}$	78.20±5.73$^{\#\#}$	313.0±20.8$^{\#\#}$	0.21±0.02$^{\#\#}$
Group 6	35.30±3.19$^{\#\#}$	77.80±5.28$^{\#\#}$	330.0±23.2$^{\#\#}$	0.20±0.02$^{\#\#}$
Group 7	34.30±3.24$^{\#\#}$	76.10±5.58$^{\#\#}$	359.0±27.9$^{\#}$	0.17±0.01$^{\#\#}$
F	9.73$^{\$}$	8.66$^{\$}$	9.73$^{\$}$	24.00$^{\$}$

Data are expressed as mean±SE; n=6. Significant P value ATDs *vs.* control **<0.01; Therapy *vs.* ATDs $^{\#}$<0.05; $^{\#\#}$<0.01 for Student's *t* test. Significant ANOVA at $P<0.05^{\$}$.

Group 1: control; Group 2: *P. daemia per se* (400 mg/kg); Group 3: Anti tuberculosis drugs; Group 4: ATDs + *P. daemia* 100 mg/kg; Group 5: ATDs + *P. daemia* 200 mg/kg; Group 6: ATDs + *P. daemia* 400 mg/kg; Group 7: ATDs + Silymarin (50 mg/kg); ALT: Alanine aminotransferase; AST: Aspartate aminotranferase; ALP: Alkaline phosphatase.

The ATDs treated animals showed significant elevation in triglycerides and cholesterol in serum when compared to control

group (P<0.01). *P. daemia* reduced the elevated level of triglycerides and cholesterol at 100, 200 and 400 mg/kg doses (P<0.01). Both 200 and 400 mg/kg doses showed restoration in glucose towards control (P<0.01); however, 100 mg/kg dose was significant at P<0.05. Significant decrease in albumin (P<0.01) after ATDs administration was restored by *P. daemia* (200 mg/kg) treatment (P<0.05) (Table 2).

Table 2

Therapeutic influence of *P. daemia* extract against anti-tuberculosis drugs induced toxicity in serum biochemistry.

Groups	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Albumin (g/dL)	Glucose (mg/dL)
Group 1	36.40±2.53	14.30±1.14	4.51±0.34	101.00±7.89
Group 2	30.10±2.77	17.30±1.08	4.46±0.35	94.80±7.03
Group 3	86.90±5.98**	47.70±3.99**	3.13±0.20**	69.70±4.54**
Group 4	46.30±2.87$^{\#\#}$	34.40±1.99$^{\#}$	3.40±0.20	95.10±6.47$^{\#}$
Group 5	37.70±2.84$^{\#\#}$	30.70±2.44$^{\#\#}$	3.84±0.20$^{\#\#}$	97.40±6.59$^{\#\#}$
Group 6	32.10±2.80$^{\#\#}$	30.70±1.95$^{\#\#}$	3.36±0.21	100.00±7.45$^{\#\#}$
Group 7	40.30±2.73$^{\#\#}$	19.30±1.64$^{\#\#}$	4.02±0.32$^{\#}$	98.20±6.59$^{\#\#}$
F	39.50$^{\$}$	9.73$^{\$}$	5.03$^{\$}$	3.21$^{\$}$

Data are expressed as mean±SE; n=6. Significant P value ATDs *vs.* control **<0.01; Therapy *vs.* ATDs $^{\#}$<0.05; $^{\#\#}$<0.01 for Student's t test. Significant ANOVA at P<0.05$^{\$}$.

Group 1: control; Group 2: *P. daemia per se* (400 mg/kg); Group 3: Anti tuberculosis drugs; Group 4: ATDs + *P. daemia* 100 mg/kg; Group 5: ATDs + *P. daemia* 200 mg/kg; Group 6: ATDs + *P. daemia* 400 mg/kg; Group 7: ATDs + Silymarin (50 mg/kg).

3.2. Oxidative stress and aniline hydroxylase activity

Significant diminution in GSH contents was observed after eight weeks of ATDs administration. *P. daemia* extract restored GSH at all doses (P<0.01, F=11.70) (Figure 1A). Significantly increased lipid peroxidation in liver as well as microsomes was observed in terms of enhanced TBARS in ATDs treated rats (P<0.01, lipid peroxidation: F=66.70; microsomes lipid peroxidation: F=39.20). All the three doses of *P. daemia* significantly decreased LPO (P<0.01) when compared to control; however, only 200 and 400 mg/kg doses diminished microsomal LPO (P<0.01) (Figure 1B-C). Activity of aniline hydroxylase in microsomal fraction was significantly up regulated in ATDs administered animals (P<0.01, F=9.27). Treatment with *P. daemia* at 200 and 400 mg/kg doses down regulated aniline hydroxylase activity towards control (P<0.01), whereas 100 mg/kg dose revealed significant reversal (P<0.05) (Figure 1D).

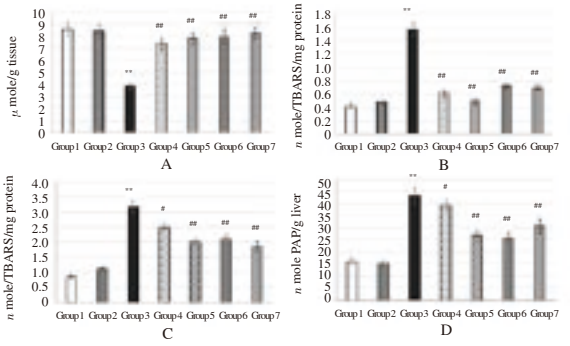

Figure 1. Therapeutic effect of *P. daemia* on antioxidant status, oxidative stress and aniline hydroxylase activity against ATDs induced toxicity. Data are expressed as mean±SE; n=6; Significant P value ATDs *vs.* control $^{*}P$<0.05; $^{**}P$<0.01; Therapy *vs.* ATDs $^{\#}P$< 0.05; $^{\#\#}P$<0.01 for Student's t test. A: Glutathione; B: Lipid peroxidation; C: Microsomal lipid peroxidation; D: Aniline hydroxylase.

Group 1: Control; Group 2: *P. daemia per se* (400 mg/kg); Group 3: Anti-tuberculosis drugs (ATDs); Group 4: ATDs + *P. daemia* 100 mg/kg; Group 5: ATDs + *P. daemia* 200 mg/kg; Group 6: ATDs + *P. daemia* 400 mg/kg; Group 7: ATDs + Silymarin 50 mg/kg.

3.3. Enzymatic antioxidant status

Significant reduction (P<0.01) was seen in catalase and SOD activity after exposure to ATDs for eight weeks (Figure 2). Analysis of variance indicated significant recovery in level of catalase at all the three doses of *P. daemia* treatment (F=16.5); however, 200 and 400 mg/kg doses were found to be more effective (P<0.01) when compared to 100 mg/kg dose (Figure 2A). The SOD level was restored closer to control significantly at 100, 200 mg/kg and 400 mg/kg doses (P<0.01, F=11.1) (Figure 2B). Enzymatic antioxidant status indicated that 200 and 400 mg/kg dose of *P. daemia* was almost equally effective and may be used for further studies. No adverse effects were observed on activity of enzymatic antioxidants after *per se* treatment.

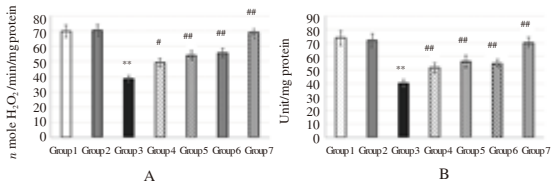

Figure 2. Therapeutic effect of *P. daemia* on enzymatic antioxidant status against ATDs induced toxicity.

Data are expressed as mean±SE; n=6; Significant P value ATDs *vs.* control $^{*}P$<0.05; $^{**}P$<0.01; Therapy *vs.* ATDs $^{\#}P$<0.05; $^{\#\#}P$<0.01 for Student's t test.

A: Catalase; B: Superoxide dismutase.

Group 1: Control; Group 2: *P. daemia per se* (400 mg/kg); Group 3: Anti-tuberculosis drugs (ATDs); Group 4: ATDs + *P. daemia* 100 mg/kg; Group 5: ATDs + *P. daemia* 200 mg/kg; Group 6: ATDs + *P. daemia* 400 mg/kg; Group 7: ATDs + Silymarin 50 mg/kg.

3.4. GSH cycle

Activity of GPx, GR and G6PDH was significantly decreased after ATDs exposure for eight weeks (Figure 3A-C). Activity of GPx (F=10.6), GR (F=12.9) and G6PDH (F=18.1) showed significant recovery at all doses of *P. daemia* (P<0.01). *P. daemia* extract at 200 and 400 mg/kg doses depicted better effects than 100 mg/kg dose.

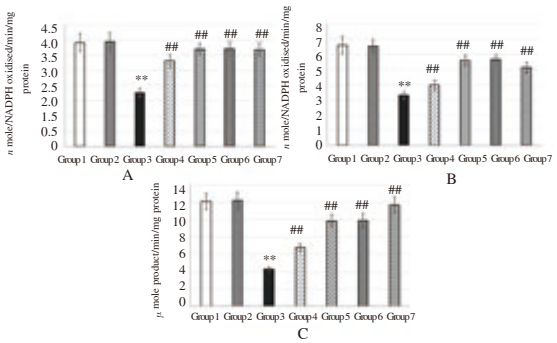

Figure 3. Therapeutic effect of *P. daemia* on GSH cycle against ATDs induced toxicity.

Data are expressed as mean±SE; n=6; Significant P value ATDs *vs.* control *P<0.05; **P<0.01; Therapy *vs.* ATDs $^{\#}P$<0.05; $^{\#\#}P$<0.01 for Student's t test. A: Glutathione peroxidase; B: Glutathione peroxidase; C: G6PDH. Group 1: Control; Group 2: *P. daemia per se* (400 mg/kg); Group 3: Anti-tuberculosis drugs (ATDs); Group 4: ATDs + *P. daemia* 100 mg/kg; Group 5: ATDs + *P. daemia* 200 mg/kg; Group 6: ATDs + *P. daemia* 400 mg/kg; Group 7: ATDs + Silymarin 50 mg/kg; G6PDH: Glucose-6-phosphate dehydrogenase.

3.5. Tissue biochemical observations

Concentration of triglycerides and cholesterol was significantly increased (P<0.01) after administration of ATDs. *P. daemia* therapy showed protective effect by reducing triglycerides (F=21.6) and cholesterol (F=26.1) (P<0.01) (Figure 4A-B). The ATDs administration significantly decreased (P<0.01) total protein content in liver and hepatic microsomes (Figure 4C-D). The *P. daemia* therapy restored protein contents and doses of 200 and 400 mg/kg showed maximum recovery ((Hepatic protein: F=3.76; Microsomal protein: F=5.82; P<0.05).

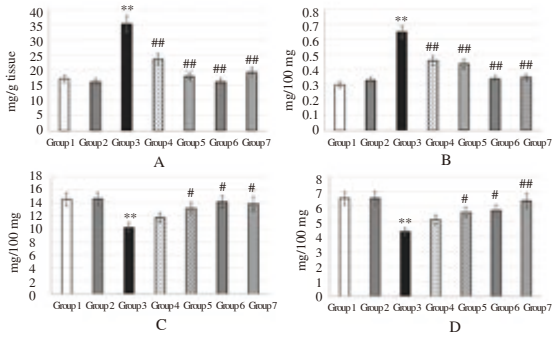

Figure 4. Therapeutic effect of *P. daemia* on tissue biochemical parameters against ATDs induced toxicity.

Data are expressed as mean±SE; n=6; Significant P value ATDs *vs.* control *P<0.05; **P<0.01; Therapy *vs.* ATDs $^{\#}P$<0.05; $^{\#\#}P$<0.01 for Student's t test. A: Triglycerides; B: Cholesterol; C: Hepatic protein; D: Microsomal protein. Group 1: Control; Group 2: *P. daemia per se* (400 mg/kg); Group 3: Anti-tuberculosis drugs (ATDs); Group 4: ATDs + *P. daemia* 100 mg/kg; Group 5: ATDs + *P. daemia* 200 mg/kg; Group 6: ATDs + *P. daemia* 400 mg/kg; Group 7: ATDs + Silymarin 50 mg/kg.

3.6. Histopathological observations

Photomicrograph of control liver showed regular histoarchitecture with well-defined central vein, sinusoidal space and cordially arranged hepatocytes with rounded nucleus. Exposure to ATDs disturbed cord arrangement of hepatocytes with appearance of pycknotic nuclei and scanty sinusoidal spaces. Administration of *P. daemia* at 100, 200 and 400 mg/kg dose recovered histoarchitecture towards control (Figure 5).

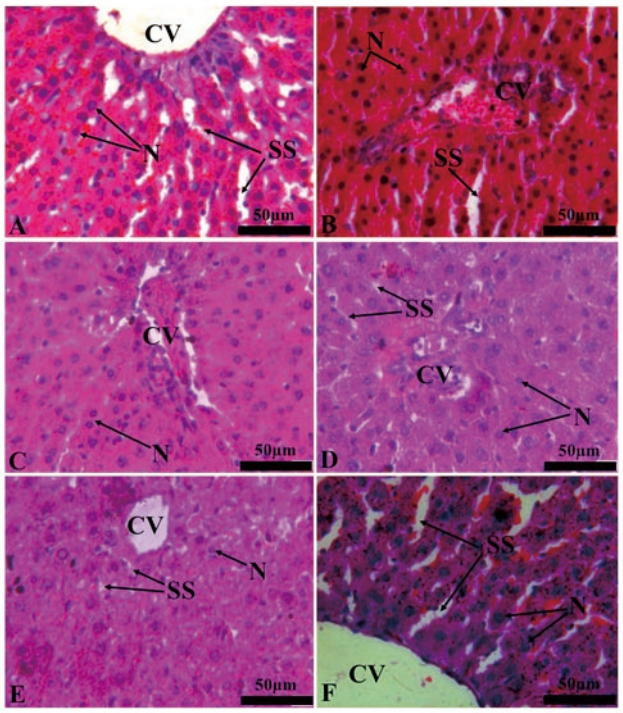

Figure 5. Therapeutic effect of *P. daemia* on histological alterations induced by ATDs.

A: Liver of control rat showed normal arrangement of hepatocytes, central vein (CV), sinusoidal space (SS) and rounded nucleus (N) (400×). B: ATDs intoxicated group showed enlarged hepatocytes with loss of cordial arrangement, disturbed sinusoidal space, vacuolation (V), inflammatory cell infiltration and a few hepatocytes with centrally located nucleus (400×). C):Therapy of *P. daemia* at 100 mg/kg showed mild hepatocyte vacuolization (V) and relatively less alteration in central vein and sinusoidal space (400 ×). D: Treatment of *P. daemia* at 200 mg/kg showed significant protection with negligible inflammation in hepatocytes and rounded nucleus (400×). E: *P. daemia* at 400 mg/kg treated group depicted rounded nucleus, cordially arranged hepatocytes and well-formed central vein (400×). F: Silymarin at 50 mg/kg treated group showed normal and cordially arranged hepatocytes around central vein (400×).

3.7. Transmission electron microscopic observations

Ultra structural observations of hepatocyte of control animal showed regular appearance of mitochondria and cytoplasmic density. ATDs exposure caused cellular damage with cytoplasmic

vacuolation, mitochondrial degeneration that was conserved by *P. daemia* therapy at dose of 200 mg/kg (Figure 6).

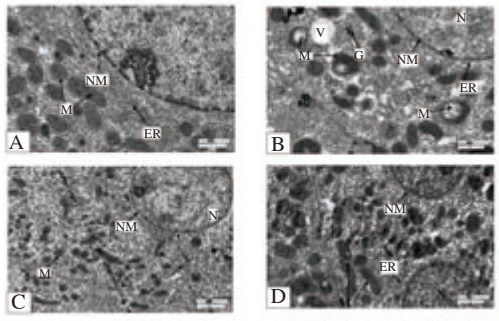

Figure 6. Therapeutic effect of *P. daemia* on ultra-structural alterations induced by ATDs.

A: Electron micrograph of liver from control rat showed regular appearance of mitochondria (M) and cytoplasmic density (2 550×). B: ATDs exposure caused cellular damage with cytoplasmic vacuolation (V), mitochondrial degeneration, randomly scattered endoplasmic reticulum (ER) and glycogen (G) (4 000×). C: Liver cells of rats treated with ATDs and *P. daemia* at 200 mg/kg dose showed normal nucleus, well-fenestrated mitochondrion, well distributed endoplasmic reticulum, dense and evenly distributed cytoplasm with lesser vacuolation (1 550×). D: Liver cells of rats treated with ATDs and silymarine at 50 mg/kg showed almost normal appearance with minimal cellular deterioration (2 550×).

4. Discussion

Adverse effects of allopathic drugs are responsible for about 20%-40% cases of liver failure[32]. Oxidative stress due to formation of reactive metabolites like hydrazine of ATDs is a major cause of drug induced hepato-renal toxicity[9,33]. Plant derived antioxidants are well known as an alternative source to promote disease resistant ability[34]. Purpose of this study was intended to report potential of *P. daemia* as a protective agent against ATDs induced liver damage.

The most common cause of hepatic injury after ATDs treatment is INH toxicity[35]. The ATDs induced destruction of liver is extrapolated on the basis of alterations in relevant biomarkers[36]. Exposure to INH caused generation of free radicals, which hempered membrane integrity of the hepatocytes and favored leakage of enzymes in systemic circulation[37,38]. Administration of *P. daemia* extract prevented ATDs induced deviations in hepatic biomarkers due to its antioxidant potential that could maintain the cellular integrity[39].

Cholesterol and triglycerides play vital role in construction of cell membranes and production of essential hormones. Elevated level of cholesterol often occurs in biliary blockade and may touch to a toxic level. Following ATDs administration, elevated serum cholesterol and triglycerides with reduction in glucose indicated disturbance in lipid metabolism[40,41]. Inhibition in bile synthesis from cholesterol due to ATDs toxicity could be responsible for increased level of cholesterol which got reversed towards control by bioactive components of *P. daemia* as found in case of CCl_4 intoxication[12]. Significant reversal in glucose level was noted in rats treated with *P. daemia* extracts, which might be due to the improvement in carbohydrate metabolism. Terpenoids present in *P. daemia* strengthen the concentration of antioxidants in injured area and could restore inflamed tissues by increasing blood supply[42].

Hepatic cytochrome P450 enzymes are responsible for metabolic bioactivation of wide variety of xenobiotics, including isoniazid and rifampicin[35]. Primary pathway of isoniazid metabolism includes acetylation in liver to form isonicotinic acid and monoacetyl hydrazine, which is oxidized by CYP2E1 to form reactive acylated toxic species[43,44]. Significant induction was seen in marker of CYP2E1 *i.e.*, aniline hydroxylase activity after exposure to ATDs in this study. The findings suggest significant role of CYP2E1 and its marker aniline hydroxylase exists in adaptive response against microsomal enzyme dependent oxidative stress induced by INH and RIF. Inhibition of CYP2E1 with antioxidant effect of herbal remedies and isolated natural compounds may be beneficial for liver protection against ATDs that induced cellular damage[45,46]. Flavonoids and their derivatives are metabolized by CYP2E1 and inhibit their activity[47,48]. The curative potential of *P. daemia* due to presence of bioactive flavonoids as major phytochemicals found in its leaves cannot be ignored[49]. Polyherbal formulation (Heptoplus™) also regulate CYP2E1 activity which also favors of findings of present study[50].

Oxidative stress implicated in terms of enhanced TBARS and protein oxidation with concomitant decrease in GSH after ATDs exposure[33]. Massive production of reactive metabolite of ATDs makes large amount of free radicals unbound due to GSH depletion[51,52]. Free radicals form covalent binding with macromolecules on cellular proteins which seems to start peroxidative damage of membrane lipids and endoplasmic reticulum[53,54] which also result in decreased protein content and increased protein oxidation[55]. Elevated level of LPO after ATDs toxicity also suggests failure of antioxidant defense mechanism[56]. Herbal products are effective antioxidant due to presence of their polyphenolic components. Treatment of *P. daemia* extract diminished LPO and protein oxidation and maintained antioxidant homeostasis near to control. Flavonoids and tannins present in extract of *P. daemia* could donate hydrogen atom and possess electrophile scavenging property responsible for its therapeutic activity[39].

Reactive oxygen species are primarily eliminated by superoxide dismutase and catalase[57]. Results obtained here signify that ATDs induced significant decrease in SOD and catalase because of excessive formation of superoxide anion, which inactivated H_2O_2 dependent enzymes catalase and GPx[53]. Administration of *P. daemia* extract significantly improved levels of SOD and catalase possibly due to defense mechanism of its flavonoids involved in free radical scavenging activity[39]. Interpretations made in favor of findings of this study are based on previous reports on therapeutic effects of ethanolic extract of *Solanum xanthocarpum* against INH and RIF induced hepatotoxicity[58]. The GSH redox cycle includes GR, GPx and G6PDH. The GPx, GR and GSH efficiently scavenge toxic metabolites of ATDs. Altered activity of these enzymes after ATDs treatment could be due to impairment of antioxidant defense mechanism and GSH depletion due to overwhelming oxidative

stress[59]. Cytosolic enzyme GR catalyze reduction of GSSG (end product of GPx activity) to GSH. The ATDs toxicity caused marked reduction in GPx activity leading to insufficient substrate availability to GR, thereby hampering its normal activity[60]. Both GSH and GPx play a crucial role in maintenance of membrane integrity, enzyme activity and detoxification capacity of biological systems[61]. Exogenous antioxidants are involved in modulation of GSH metabolism[62]. Administration of *P. daemia* restored activity of GR and accelerated utilization of GSSG to form GSH. Thus, restoration of GSH subsequently elevated activity of G6PDH, GPx and GR. Similar findings have been reported on hepatoprotective activity *Ginkgoselect* Phytosome® against rifampicin induced toxicity[53].

Histoarchitectural and ultrastructure observations revealed changes in liver structure in ATDs intoxicated group and indicated disturbed structural and functional integrity of the cells[2,63]. Relatively less evidences of cellular damage and necrosis was observed in *P. daemia* treated groups.

The present study suggests that *P. daemia* extract at doses of 200 and 400 mg/kg was found to be effective in combating liver injury induced due to oxidative stress. Findings of this study provide evidence in support of medicinal value of *P. daemia* that may be used to alleviate ATDs induced liver injury.

Conflict of interest statement

The authors declare no conflict of interest on any issue.

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