Naringenin protects hepato-renal tissues against antituberculosis drugs induced toxic manifestations by modulating IL-6, IGF-1, CYP2E1, biochemical and ultra-structural integrity

Nisha Sahu

Guru Ghasidas University

Samrat Rakshit

Guru Ghasidas University

Satendra Kumar Nirala

Guru Ghasidas University

Monika Bhadauria (✉ monikabhadauria@rediffmail.com)

Guru Ghasidas University

Research Article

Keywords: Antituberculosis drugs, Naringenin, Hepatorenal injury, Ultra morphology, Oxidative stress, IL-6, IGF-1

Posted Date: March 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-934724/v2>

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Abstract

The antituberculosis drugs (ATDs), isoniazid, rifampicin, pyrazinamide and ethambutol prompt extreme hepatic and renal damage during treatment of tuberculosis. The present study aimed to investigate protective potential of naringenin against ATDs induced hepato-renal injury. Rats were administered with ATDs orally for 8 weeks (3 days/week) followed by naringenin at three different doses (10, 20 and 40 mg/kg) conjointly for 8 weeks (3 days/week alternately to ATDs administration) and silymarin (50 mg/kg) as positive control. Exposure to ATDs caused significant increase in IL-6, triglycerides, cholesterol, bilirubin whereas depletion in IGF-1, albumin and glucose in serum. Endogenous antioxidants GR, GPx and G-6-PDH were diminished in liver and kidney tissues with parallel increase in triglycerides, cholesterol, microsomal LPO and aniline hydroxylase (CYP2E1 enzyme). Ultra-structural observations of liver and kidney showed marked deviation in plasma membranes of various cellular and sub-cellular organelles after 8 weeks of exposure to ATDs. Conjoint treatment of naringenin counteracted ATDs induced toxic manifestations by regulating IL-6, IGF-1, CYP2E1, biochemical and ultra-structural integrity in a dose dependent manner. Naringenin has excellent potential to protect ATDs induced hepato-renal injury by altering oxidative stress, modulation of antioxidant enzymes, serum cytokines and ultra-structural changes.

Introduction

Tuberculosis is one of the top ten causes of death worldwide (1). The first-line anti tuberculosis drugs (ATDs), isoniazid, pyrazinamide, rifampicin and ethambutol are prescribed to patients as single tablet in the first phase for 2 months and in combination (isoniazid+rifampicin) in the second phase for another 4 months (2). Therapy of rifampicin and pyrazinamide increases isoniazid induced liver injury (3). Adverse drug reactions (ADRs) due to ATDs may cause skin reaction, gastrointestinal, neurological disorders (4), hepatotoxicity (5), nephrotoxicity or even death, if not diagnosed and treated timely (6). Liver in association with kidney performs metabolism and biotransformation and the kidney always remains at risk of injury through continuous exposure to high concentration of xenobiotics (7). Reactive metabolites so evolved weaken the antioxidant defense of the cell resulting tissue injury (8). Control on tuberculosis without posing side effect of ATDs is still a challenge to clinicians. Search for alternative and/ or complementary therapeutics may provide protection from ATDs induced toxic manifestations in liver and kidney.

Nature is rich with diversified natural compounds that may be explored for potential drugs. Naringenin is a plant bioflavonoid, mainly found in grapes, tomatoes and citrus fruits and profoundly helpful to human well beings due to its inherent antioxidant potential and excellent bioavailability (9). Its chemical structure has polyphenolic compound with two benzene rings connected together with a heterocyclic pyrone ring (10) that makes it good antioxidant. It is medicinally used to treat osteoporosis, growth and cardiovascular diseases and also has lipid lowering and insulin-like properties, anti-tumor (11), anti-viral (12), anti-diabetic (13), anti-inflammatory (14), hepatoprotective, nephroprotective (15), antioxidant (16), immunomodulatory (17) and neuroprotective properties (18).

Our research group previously reported that therapy of naringenin maintains level of biomarkers of liver and kidney function tests, decreases lipid peroxidation, increases glutathione content and activities of catalase and superoxide dismutase and decreases histological lesions (19). On the basis of that report, it was hypothesized that naringenin therapy may improve activity of enzymes glutathione reductase (GR) and glutathione peroxidase (GPx), which involve in maintaining homeostasis between GSH and GSSG. Thus, activities of GR, GPx and glutathione-6-phosphate dehydrogenase had been assessed in this study. Among numerous cytokines relevant to DILI susceptibility and progression, there is compelling evidence that Interleukin-6 (IL-6) plays an important role in acute phase response, liver regeneration and protection against liver injury by a variety of drugs (Wang et al., 2015). Insulin like growth factor-1 (IGF-1) is produced primarily by the liver as an endocrine hormone and stimulates growth-promoting effects on almost every cell as well as regulates cellular DNA synthesis. Both IL-6 and IGF-1 were chosen to assess protective role of naringenin on inflammatory cytokines. Biotransformation mainly takes place in endoplasmic reticulum and enzymes of CYP2E1 family are involved in the biotransformation of ATDs. Thus, CYP2E1 in terms of aniline hydroxylase was also determined. Synthesis and storage activity of liver in terms of albumin, total protein, triglyceride and cholesterol were taken into consideration and determined in serum and tissues both.

Thus, present study was undertaken in support of curative effect of naringenin against anti-tuberculosis drugs induced hepato-renal injury considering endogenous antioxidant enzyme, CYP2E1 activity, synthesis and storage activities, inflammatory aspects and electron microscopy.

Materials And Methods

Chemicals

The ATDs (isoniazid, rifampicin, ethambutol and pyrazinamide) were generously obtained from the Government TB hospital, Bilaspur (C.G.). Naringenin and all other chemicals were of pure and analytical grade and procured from standard chemical dealers.

Animals

Healthy female albino Wistar strain rats weighing 150 ± 10 g body weight was used for the study. The animals were housed under standard husbandry conditions (temperature 25 ± 2 °C temp, relative humidity 62-72%, light-dark cycle of 14 h and 10 h) and fed on standard pelleted diet and drinking water *ad libitum*.

Study design

Animals were divided into seven groups of six animals each. Group I was treated with vehicle and served as control. Group II was administered with naringenin per se at the dose of 40 mg/kg orally. Group III to VII were administered with four ATDs namely pyrazinamide, ethambutol, isoniazid and rifampicin with 1 h of break in between each at the doses of 210, 170, 85 and 65 mg/kg respectively for 8 weeks (3 alternate

days in a week) and group III served as experimental control. Animals of groups IV, V and VI were given naringenin at the doses of 10, 20 and 40 mg/kg, p.o., respectively for 8 weeks (3 alternate days in a week considering every next day to ATD exposure). Group VII was treated with silymarin at dose of 50 mg/kg, p.o., as positive control. All animals were euthanized after 24 h of the last treatment; blood, liver and kidney were taken out from the animals and stored at -20°C until analyzed.

Serum biochemical assays

The serum was used for determination of albumin, triglycerides, cholesterol and blood sugar level using diagnostic kits according to the manufacturer's instructions (Erba diagnostics, Germany).

Aniline hydroxylase for CYP2E1 activity

Aniline hydroxylase (AH) was determined by assessing intensity of blue colored conjugate of phenol with p-amino phenol (PAP) (20). Microsomal lipid peroxidation (21) and microsomal protein (22) were also determined.

Tissue biochemical assays

Hepatic and renal tissues were processed to determine cholesterol (23), triglycerides (24) and total proteins (22).

Antioxidant enzyme activity

The activities of glutathione peroxidase was analyzed by measuring the reduction of NADP⁺ to NADPH (25), glutathione reductase was weighed by measuring the amount of NADPH utilized (26) and glucose-6-phosphatase dehydrogenase was assessed by measuring the rate of conversion of NADPH to NADP⁺ (27).

Interleukin-6 and insulin like growth factor-1 in serum

Cytokineinterlukin-6 (IL-6) and insulin like growth factor-1 (IGF-1) were assessed in serum using ELISA based kits (Ray Biotech, INC, Norcross, GA 30092 USA) and the results were expressed as pg/ml. Entire procedures for assessment were performed following instruction manual of diagnostic kits.

Electron microscopy examination

About 1 mm³ pieces of liver and kidney samples were processed for Transmission electron microscopy (28).

Statistical analyses

The results were presented as mean $\pm$ SE. One-way analysis of variance (ANOVA) were used followed by Tukey's *post hoc* analysis of honestly significant difference (HSD) at $P \leq 0.05$ to draw a statistical

comparison among different treatment groups (29). Microsoft office was used to calculate ANOVA, Tukey's *post hoc* HSD test and percent protection. Following formula was used to calculate percent protection (30)

$$\left(1 - \frac{\left[\frac{\text{(Therapy-Control)}}{\text{(Toxicant-Control)}}\right]}{\left[\frac{\text{(Therapy-Control)}}{\text{(Toxicant-Control)}}\right]}\right) \times 100$$

Results

Serum biochemical parameter

Exposure to ATDs for 8 weeks significantly elevated ($P \leq 0.01$) level of triglyceride, cholesterol and bilirubin, whereas significantly declined albumin and glucose ($P \leq 0.01$) (Table 1) as compared with control group. Treatment with naringenin at three different doses (10, 20 and 40 mg/kg) maintained biochemical variables towards control. Higher doses, 20 and 40 mg/kg of naringenin significantly maintained all the serological variables towards control. Tukey's HSD *post hoc* test revealed that 40 mg/kg dose of naringenin caused significant difference in comparison to 10 and 20 mg/kg dose and showed better protective effect. Treatment with naringenin at 40 mg/kg dose offered more than 90% protection in glucose and bilirubin level as well minimum 53% protection in albumin, triglycerides and approximately 66% protection in cholesterol level indicating improvement against ATDs toxicity. Efficacy of naringenin was well compared to silymarin positive control group and results clearly showed protective potential of naringenin at par silymarin.

Aniline hydroxylase for CYP2E1 activity

Oral administration of ATDs over a period of 8 weeks increased activity of drug metabolizing enzyme, aniline hydroxylase (Fig. 1A) and microsomal lipid peroxidation (Fig. 1B) along with decline in microsomal protein (Fig. 1C). The protein content was significantly decreased while LPO and CYP2E1 activity was increased dramatically. Treatment with naringenin markedly reversed these changes towards their respective control in dose dependent manner. CYP2E1 activity was protected by naringenin in dose dependent manner with 82% protection by 40 mg/kg dose. Naringenin at 20 and 40 mg/kg dose were able to maintain microsomal LPO and protein, significant difference was observed between both the therapeutic group as revealed by Tukey's *post hoc* HSD test. The 40 mg/kg dose of naringenin show 88% and 85% protection in maintaining microsomal LPO and protein respectively.

Tissue lipid profile

Level of cholesterol and triglyceride was found to be significantly increased ($P \leq 0.05$) in liver and kidney along with decline in protein after administration of ATDs for 8 weeks as compared to control group (Figure 2). Tukey's *post hoc* HSD test revealed that 40 mg/kg dose of naringenin treated groups was able to maintain triglycerides, cholesterol and protein in hepatorenal tissue better than the lowest dose. Values of control and 40 mg/kg dose of naringenin were very similar. Percent protection analysis confirmed 40

mg/kg dose of naringenin provide approximately 93% protection to hepatic and 61% protection to renal cholesterol. The 40 mg/kg dose of naringenin effective as it provides above 94% protection to hepatorenal triglycerides and approximately more than 84% protection in maintaining hepatorenal protein respectively. The efficacy of naringenin remained at par the efficacy of silymarin treatment.

Antioxidant status

Figure 3 (A-C) illustrates activity of antioxidant enzymes namely glutathione reductase (GR), glutathione peroxidase (GPx) and glucose-6-phosphate dehydrogenase (G6PD) in liver and kidney of various treatment groups. The ATDs administration caused significant ($P \leq 0.05$) inhibition in the activities of the GR, GPx and G-6-PDH in liver and kidney. Treatment with naringenin significantly up regulated activities of these enzymes in liver and kidney ($P \leq 0.05$) in dose dependent manner and maintained enzymatic antioxidant integrity towards control. Tukey's *post hoc* HSD test revealed that, naringenin at 20 and 40 mg/kg dose showed better protective efficacy than 10 mg /kg dose in increasing hepatorenal GR, GPx and G-6-PDH. Percent protection analysis suggested that naringenin at 40 mg/kg dose showed more than 86% and 74% protection in maintaining hepatic and renal GR. Dose of naringenin 40 mg/kg dose provided more than 93% and 86% protection in maintaining hepatic and renal GPx. However, 40 mg/kg dose showed restoration of hepatorenal G-6-PDH by providing more than 95% protection. Results obtained from naringenin treated groups were found very close to silymarin treated group.

Biochemical findings indicated that 40 mg/kg dose of naringenin maximally protected from ATDs induced organ damage. Thus, only group of 40 mg/kg dose of naringenin was carried forward to examine IL-6 and IGF-1 in serum as well as electron microscopy of liver and kidney samples.

Interleukin-6 and insulin like growth factor-1 in serum

There was significant elevation in IL-6 level and decrease in IGF-1 level in serum after ATDs intoxication (Fig. 4A-B). Dose of naringenin at 40 mg/kg significantly restored these variables towards their respective control ($P \leq 0.05$) that was well compared with silymarin treated positive control group. Tukey's *post hoc* HSD test revealed that, naringenin at 40 mg/kg dose showed more than 91% protection in maintaining IL-6 level in serum and approximately 56% protection to serum IGF-1.

Electron microscopy observation

Electron microscopy of liver of control group showed well defined hepatocytes parted by canaliculi and sinusoids. Hepatocytes had nucleus with regular shape, distinct nuclear membrane, extended endoplasmic reticulum (ER) and numerous mitochondria of various shapes and size (Fig 5A). Ultra-structural observation of liver of ATDs administered group showed irregularly shaped distorted mitochondria (DM), loss of organization of endoplasmic reticulum and abundance of lipid droplets (LD) (Fig. 5B and C). Treatment with naringenin (40 mg/kg) showed better formed mitochondria, well-formed nucleus with intact nuclear membrane and obvious nucleolus, heterochromatin, euchromatin and nuclear pore; extensive endoplasmic reticulum, glycogen rosettes and scanty lipid droplets (Fig. 5D and E).

Treatment with silymarin in positive control group showed well-formed mitochondria arranged between plasma membrane undulations, nucleus with intact nuclear membrane, obvious nuclear pore, scattered heterochromatin and euchromatin; extensive endoplasmic reticulum and scanty lipid droplets (Fig. 5F).

Ultra-structure of kidney of control group showed well developed elongated mitochondria, nucleus with nuclear membrane, well-formed plasma membrane with extended convolutions (Fig. 6A). Electron micrograph of kidney of ATDs administered group showed renal damage characterized by dilated plasma membrane, irregular and degenerated undulations of plasma membrane and diffusely arranged mitochondria (Fig. 6B and C). Treatment with naringenin (40 mg/kg) preserved better formed mitochondria arranged between undulation of plasma membrane, and well-formed intact nuclei (Fig. 6D and E). Positive control group, treated with silymarin also showed similar effects as exerted by naringenin. Observations indicated well-formed nucleus with intact nuclear membrane, heterochromatin and euchromatin, well-formed plasma membrane and mitochondria of different shape and size (Fig. 6F).

Discussion

Naringenin, a flavonoid, has attracted the researchers from the last few decades due to its good bioavailability, antioxidant and anti-inflammatory activities and low toxicity (31, 32). The present study was carried out to explore possibility of naringenin as effective complementary/ therapeutic agent against ATDs induced hepato-renal toxic manifestations.

Administration of isoniazid, pyrazinamide, rifampicin and ethambutol individually or in combination causes various metabolic and morphologic changes in tissues particularly in liver as it is the main site of biotransformation (33). Any imbalance in the activity of drug metabolizing enzymes shifts equilibrium towards free radical generation that lead to damage DNA to cause mutation, lipid to cause membrane damage, or proteins to alter their activities (34). Combination of isoniazid and rifampicin induces oxidative stress to cause hepatic and renal injury ((35). Several endogenous antioxidant enzymes play role to prevent and neutralize free radical induced damage. The glutathione reductase reduces oxidized glutathione (GSSH) to reduced glutathione (GSH). Selenoenzyme, glutathione peroxidase reduces H_2O_2 and hydroperoxide to non-toxic products (36). Glucose-6-phosphate dehydrogenase catalyses synthesis of riboses for nucleic acid production and is the principal intracellular source of NADPH, which keeps GSH at constant level by scavenging ROS. Toxic intermediates of ATDs bring down the activities of these antioxidant enzymes that prompt cells more susceptible to peroxidative damage (37). Flavonoids contain one or more aromatic hydroxy groups in their chemical structure, responsible for their antioxidant potential (38) and beneficial effects against many diseases (39). In this investigation, naringenin could restore antioxidant enzymes, reduced oxidative stress and protected membrane damage by scavenging free radicals with the help of three hydroxy groups present at the 4', 5, and 7 carbons in its structure (40). Observations of this study were similar to previous reports (41).

Oxidative stress (42), infection and injury (43) induce a cascade of inflammatory reactions with increased production of cytokine, the IL-6. The IL-6 plays a role in development of various diseases, including

hypoalbuminemia (44), liver damage and carcinogenesis (45). Growth hormone, produced in the anterior pituitary gland, is released into the blood stream and stimulates liver to produce IGF-1, which is a primary mediator for the effects of growth hormone (46). Both IGF1 and growth hormone act together to promote growth by exerting growth-promoting effects on skeletal muscle, cartilage, bone, liver, kidney, nerve, skin, hematopoietic, and lung cells (47) and regulate metabolic function in adults (48). In addition to insulin-like effects, IGF-1 also regulates cellular DNA synthesis (49). Oxidative stress resulted from biotransformation of ATDs increased level of IL-6 along with decrease in IGF-1. Naringenin acted as anti-inflammatory agent by reducing the production of pro-inflammatory cytokine through various mechanisms (50). Naringenin also has ability to enhance the IGF-1 receptor mediated antioxidant defense against various toxicants (51). These mechanisms brought level of IL-6 and IGF-1 towards their respective control with structural and functional improvement in hepatic and renal tissues.

Albumin, serves a wide-range of functions, including transportation of hormones and bilirubin acts as binding agent for transporting many exogenous and endogenous compounds in circulatory system (52). The ATDs induce hypersensitivity reactions with hypoalbuminemia and hypoglycemia that signify life-threatening hepatic damage (2). Rifampicin induced conjugated hyperbilirubinemia by hampering excretion of bilirubin through inhibiting bile salt exporter pump (53). Exposure to ATDs increased triglycerides and cholesterol in serum due to severe disruption in cellular metabolism and function (54). The ATDs administration causes hyperlipidemia, which could disrupt membrane fluidity (55). The ATDs disrupt hepatic and renal phospholipids and protein synthesis due to fatty accumulation in these organs (28). Thus, pathogenesis in hepatic and renal tissues could be multi-factorial, reflecting derangement in complex biosynthetic, catabolic and enzymatic pathways and lipoprotein metabolism (56). Naringenin, defended albumins, lipids and other important biomolecules from being oxidized or destroyed by oxidative stress (57) and could maintain biochemical integrity in favor of regular functioning.

Antituberculosis drugs are bio transformed by many drug metabolizing enzymes and activation in CYP2E1 is associated with susceptibility to ATD induced liver injury (Kim et al. 2012, (58). Isoniazid and its metabolite hydrazine mainly induce enzymes of CYP2E1 group (59) that severely cause hepatotoxicity. Enhanced activity of aniline hydroxylase led to excessive buildup of reactive metabolites within the hepato-renal tissues, which favored an oxidative stress in these organs. Damage to cellular endoplasmic reticulum and mitochondria due to reactive metabolites of ATDs further disrupted drug metabolizing ability of the liver and kidney. Naringenin could suppress oxidative stress through up regulating glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase and inhibition in microsomal lipid peroxidation, which ultimately helped in modulation of drug metabolizing enzymes of CYP2E1 group. Diminished oxidative stress could also lead to integrity of other studied cellular biochemical endpoints.

Deviation from regular level of hepato-renal marker enzymes signifies damage in structural integrity of liver and kidney tissues as these enzymes are cytoplasmic in location and released into circulation as indicator of cellular damage (60). Exposure to ATDs has been reported to induce hepatic and renal injury by disrupting several cellular features (61). In this study, naringenin maintained ultra-structural integrity of

liver and kidney tissues through reducing stress on mitochondria, endoplasmic reticulum and nucleus; thus, showed healing effects by suppressing inflammation and cellular senescence, regulating DNA synthesis, improving cellular growth or regeneration in hepatic and renal tissues (62) similar to previous reports (16).

Conclusion

We concluded that naringenin at doses of 20 and 40 mg/kg has potential to protect hepato-renal tissues from antituberculosis drugs induced toxic manifestations through modulating IL-6, IGF-1, CYP2E1, biochemical and ultra-structural integrity and may further be verified using more advanced endpoints to affirm this fact.

Declarations

Funding: The research work was financially supported by University Grants Commission, New Delhi [(F42-520/2013(SR) dated 23-03-2013] sanctioned to Dr. Monika Bhadauria.

Acknowledgments: Chhattisgarh Institute of Medical Sciences (CIMS), Bilaspur generously provided antituberculosis drugs for conducting experiments. Technical support was extended from All India Institute of Medical Sciences (AIIMS), New Delhi for electron microscopy. The first author also acknowledges Guru Ghasidas University for laboratory facility.

Conflicts of interest: The authors hold no conflict of interest on any issue from this study.

Ethical Approval: All the experiments were held in accordance with the rules set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India. All the protocols of present study were approved by institutional animal ethics committee (994/Ere/Go/06/CPCSEA).

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Tables

Table 1: Protective potential of naringenin against ATDs induced deviations in serum biochemistry

	Albumin (g/dl)	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Bilirubin (mg/dl)
Control	4.43±0.32	99.2±5.17	33.1±2.28	15.3±1.11	0.18±0.01
Naringenin <i>per se</i>	4.36±0.31	99.5±5.40	34.2±2.72	15.6±1.37	0.20±0.01
ATD	3.31±0.28 *	59.7±4.63*	74.3±4.62*	58.2±5.52*	0.43±0.03*
ATD+ Naringenin (10 mg/kg)	3.36±0.28 05%	69.1±4.13 ^a 24%	58.1±3.15 ^{aψ} 39%	43.8±2.81 ^{aψ} 34%	0.24±0.02 ^{aψ} 76%
ATD+ Naringenin (20 mg/kg)	3.73±0.27 37%	83.4±6.67 ^ψ 60%	54.2±3.36 ^ψ 49%	40.7±3.30 ^{aψ} 41%	0.22±0.02 ^ψ 84%
ATD+ Naringenin (40 mg/kg)	3.91±0.23 53%	98.3±6.37 ^{φψ} 98%	52.6±3.56 ^{aψ} 53%	29.4±2.37 ^{aλψ} 66%	0.20±0.02 ^ψ 92%
ATD+ Silymarin (50 mg/kg)	4.07±0.24 ^ψ 68%	90.5±5.45 ^{φψ} 78%	45.2±3.03 ^{aφψ} 71%	18.8±1.42 ^{φλΩψ} 92%	0.19±0.01 ^{φψ} 96%
ANOVA	6.16 [@]	15.8 [@]	36.2 [@]	103.9 [@]	57.6 [@]

Data are presented as mean ± S.E (n=6). @ represents significant value of ANOVA at 5%. * Control Vs ATD; ^Y ATD Vs ATD + Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); ^a Control Vs Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); ^j Naringenin (10 mg/kg) Vs Naringenin (20 mg/kg, 400 mg/kg) and Silymarin (50 mg/kg); ^λ Naringenin (20 mg/kg) Vs Naringenin (40 mg/kg) and Silymarin (50 mg/kg); ^Ω Naringenin (40 mg/kg) Vs Silymarin (50 mg/kg) for Tukey's HSD *post hoc* analysis at P£ 0.05. ATDs represents Antituberculosis drugs.

Figures

Figure 1

(A-C). Therapeutic effect of naringenin on microsomal lipid peroxidation, protein and aniline hydroxylase.

Data are presented as mean $\pm$ S.E (n=6). @ represents significant value of ANOVA at 5%. * Control Vs ATD; Ψ ATD Vs ATD + Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); α Control Vs Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); λ Naringenin (10 mg/kg) Vs Naringenin (20 mg/kg, 400 mg/kg) and Silymarin (50 mg/kg); Λ Naringenin (20 mg/kg) Vs Naringenin (40 mg/kg) and Silymarin (50 mg/kg); Ω Naringenin (40 mg/kg) Vs Silymarin (50 mg/kg) for Tukey's HSD *post hoc* analysis at $P \leq 0.05$. ATDs represents Antituberculosis drugs. I = Control, II = Naringenin 40 mg/kg, III = ATD, IV = ATD + Naringenin 10 mg/kg, V = ATD + Naringenin 20 mg/kg, VI = ATD + Naringenin 40 mg/kg, VII = ATD + Silymarin 50 mg/kg. F variance of CYP2E1 activity = 73.3@, microsomal lipidperoxidation= 123.3@, microsomal protein = 18.7@.

(A)

Figure 2

(A-C). Effects of ATDs and naringenin on tissue biochemistry.

Data are presented as mean $\pm$ S.E (n=6). @ represents significant value of ANOVA at 5%. * Control Vs ATD; ^Y ATD Vs ATD + Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); ^a Control Vs Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); ^j Naringenin (10 mg/kg) Vs Naringenin (20 mg/kg, 400 mg/kg) and Silymarin (50 mg/kg); ^λ Naringenin (20 mg/kg) Vs Naringenin (40

mg/kg) and Silymarin (50 mg/kg); ^Δ Naringenin (40 mg/kg) Vs Silymarin (50 mg/kg) for Tukey's HSD *post hoc* analysis at P£ 0.05. ATDs represents Antituberculosis drugs. I = Control, II = Naringenin 40 mg/kg, III = ATD, IV = ATD + Naringenin 10 mg/kg, V = ATD + Naringenin 20 mg/kg, VI = ATD + Naringenin 40 mg/kg, VII = ATD + Silymarin 50 mg/kg. F variance of Cholesterol (hepatic=30.8[@], renal=64.7[@]), triglyceride (hepatic=67.4[@], renal=55.1[@]), protein (hepatic=6.94[@], renal=7.98[@]).

Figure 3

(A-C). Protective role of naringenin on endogenous antioxidant enzymes.

Data are presented as mean $\pm$ S.E (n=6). @ represents significant value of ANOVA at 5%. * Control Vs ATD;^Y ATD Vs ATD + Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); ^a Control Vs Naringenin (10 mg/kg, 20 mg/kg, 40 mg/kg) and Silymarin (50 mg/kg); ^j Naringenin (10 mg/kg) Vs Naringenin (20 mg/kg, 400 mg/kg) and Silymarin (50 mg/kg); ^λ Naringenin (20 mg/kg) Vs Naringenin (40 mg/kg) and Silymarin (50 mg/kg); ^Ω Naringenin (40 mg/kg) Vs Silymarin (50 mg/kg) for Tukey's HSD *post hoc* analysis at P£ 0.05. ATDs represents Antituberculosis drugs. I = Control, II = Naringenin 40 mg/kg, III = ATD, IV = ATD + Naringenin 10 mg/kg, V = ATD + Naringenin 20 mg/kg, VI = ATD + Naringenin 40 mg/kg, VII = ATD + Silymarin 50 mg/kg. F variance of Glutathione reductase (hepatic=23.9[@], renal=6.58[@]), Glutathione peroxidase (hepatic=18.3[@], renal=9.31[@]), Glucose-6-phosphate dehydrogenase (hepatic=20.4[@], renal= 26.8[@]).

(A)

(B)

Figure 4

(A-B). Efficacy of naringenin on ATD induced alterations in serum IL-6 and IGF-1.

Data are presented as mean $\pm$ S.E (n=6). @ represents significant value of ANOVA at 5%. * Control Vs ATD; ^Y ATD Vs ATD + Naringenin (40 mg/kg) and Silymarin (50 mg/kg); ^Δ Naringenin (40 mg/kg) Vs Silymarin (50 mg/kg) for Tukey's HSD *post hoc* analysis at P£ 0.05. ATDs represents Antituberculosis drugs. I = Control, II = ATD, III = ATD + Naringenin 40 mg/kg, IV = ATD + Silymarin 50 mg/kg. F variance of IL-6 (24.6[@]), IGF-1 (5.12[@]).

Figure 5

(A-F): Transmission electron microscopic photographs of liver sections.

Electron micrograph of liver sample, [5(A) 1500X] of control group showing numerous regular shaped mitochondria (M) of various size as well as clusters of rough endoplasmic reticulum (ER), glycogen rosettes (G), lysosome (L), heterochromatin (Hc) and euchromatin (Eu). [5(B) 1500X] and [5(C) 2500X] of ATDs administered group for 8 weeks showing hepatic injury as lipid droplets (LD), damaged mitochondria (DM), congested nucleus (N), disintegration in cytosolic matrix, less ER, nucleus with euchromatin (Eu) are clearly visible. [5(D) 1500X] and [5(E) 1500X] of ATDs administered group for 8 weeks with 40 mg/kg dose of naringenin showing recovery as there is almost normal hepatocytes structure with normally appeared numerous mitochondria (M), integrity in cytosolic matrix, glycogen rosettes (G), heterochromatin (Hc), euchromatin (Eu), nucleolus and scattered ER are clearly visible. [5(F) 1500X] ATD administered rat for 8 weeks with 50 mg/kg dose of silymarin are well compared with control group having normal mitochondria (M), lysosomes (L), nucleus (N) and nuclear envelop with nuclear pores.

Figure 6

(A-F): Transmission electron microscopic photographs of liver sections.

Electron micrographs of kidney sample, [6(A) 1500X] of control group showing normal architecture of kidney having elongated numerous mitochondria (M) in between plasma membrane (PM) undulations. [6(B) 1500X] and [6(C) 2500X] of ATDs administered group for 8 weeks showing renal damage by presence of deformed mitochondria (M), nucleus (N) and lipid droplets (LD), degenerated undulation of

plasma membrane were also detected. [6(D) and 6(E) 1500X] of ATDs administered group for 8 weeks with 40 mg/kg dose of naringenin showing recovery towards control due to clearly visible mitochondria (M) of different shapes and size, presence of nucleus (N) with heterochromatin (Hc), euchromatin (Eu), and well-formed undulations of plasma membrane. [6(F) 1500X] of ATDs administered group for 8 weeks with 50 mg/kg dose of silymarin showing regeneration with well-developed nucleus (N), plasma membrane (PM) and presence of abundant normal mitochondria (M).