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EVALUATION OF HEPATOPROTECTIVE AND ANTI-OXIDANT ACTIVITY OF *AMARANTHUS ROXBURGHIANUS* AGAINST DRUG INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

The aim of the current study was to evaluate the hepatoprotective and anti-oxidant activity of the hydroalcoholic extract of *Amaranthus roxburghianus* against anti-tubercular drugs induced liver damage in experimental animals. The first line anti-tuberculosis drugs isoniazid (INH) and rifampicin (RIF) continues to be the effective drugs in the treatment of tuberculosis; however, the use of these drugs is associated with toxic reactions in tissues, particularly in the liver, resulting in hepatitis. *A. roxburghianus* dried aerial parts were extracted with ethanol: water (70:30) ratio, respectively, by the hot Soxhlet method. Two doses 200 and 400 mg/kg of HAAR were tested against Anti tubercular drugs -induced hepatotoxicity in albino Wistar rats. Hepatoprotective effect of silymarin was investigated by co-administration of silymarin along aside the drugs. Treatment of rats with INH+RIF induced hepatotoxicity as evidenced by biochemical measurements: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities and the levels of total cholesterol were elevated, and the total protein and total bilirubin were decreased in drugs-treated animals. Meanwhile, in vivo antioxidant activities such as SOD, CAT and MDA were increased when compared to normal group in liver homogenate also histological examinations were carried out to assess hepatoprotective activity. Histopathological changes were also observed in liver of animals that received drugs. It can be concluded that the active components of silymarin had protective effects against hepatotoxic actions of drugs utilized in the chemotherapy of tuberculosis in animal models. Since no significant toxicity of silymarin is reported in human studies, this plant extract is often used as a dietary supplement by patients taking anti-tuberculosis medications.

INTRODUCTION

Tuberculosis (TB) is one of the major communicable diseases, and nearly 2 million people die every year [1]. TB is ranked seventh among all illness [2]. Isoniazid (INH) and rifampicin (RIF) are the 2 major regimens currently used for the treatment of TB for a period of 4 to 6 months [3], which can induce hepatotoxicity [4]. The incidence of hepatic dysfunction is more, when INH and RIF are used in combination [5]. In India the prevalence of hepatotoxicity is 11.6%, when Compared to western countries where it is 4.3% [6]. Antituberculosis drug induced hepatotoxicity ranges from non-specific elevation of transaminases to fulminant of liver failure [7]. The liver dysfunction is due to the synergistic effect of INH and RIF [8]. Hydrazine (HYZ) a metabolite of INH is converted to toxic compound by CYP450, which leads to hepatotoxicity. Liver is a vibrant organ that produces and secretes bile; it also generates fundamental blood anticoagulation

factors like prothrombin, fibrinogen, and heparin. Liver transfigures sugar into glycogen [9]. Liver plays a serious role with the detoxification and excretion of many endogenous and exogenous compounds; any type of injury (due to systemic drugs, food preservatives, agrochemicals and addiction to alcohol) or impairments of its functions may lead to many complications in one's health [10]. *Amaranthus roxburghianus* is one of the traditionally well-known plant with outstanding therapeutic properties, and is used mostly in treating different diseases in India. *Amaranthus roxburghianus* is commonly, called as Prince's feather (English) chirikoora (Telugu). It is also distributed in Occasional weed of waste lands and cultivated lands [11]. The high antioxidant capacity of *Amaranthus roxburghianus* has been attributed to high levels of polyphenolic compounds specially alkaloids.

MATERIALS AND METHODS

Plant collection and authentication: A. roxburghianus plant was purchased from the local market of Tirupati, Chittoor District, Andhra Pradesh state of India. Authentication of plant was carried out by Dr. K. Madava Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati, A.P., India. A voucher specimen of the plant (Ref. No. 0829), dated 05/01/2020, has been preserved there for future references.

Extract preparation: The aerial plants were obtained by cutting the root portions and thoroughly washed with tap water, and air dried in the shade, powdered in grinder. The dried powder was extracted with ethanol: water with (70:30) by the hot Soxhlet method. The hydroalcoholic extract was concentrated in a rotary evaporator under reduced pressure. The dried crude hydroalcoholic A. roxburghianus extract (HAAR) was collected and preserved in an airtight glass container at 4–8°C until final use.

Experimental animals: Male rats of albino Wistar strain weighing between 180 - 200 g were used for the experiments. All the animals were obtained from Sree Venkateshwara Enterprises, Bengaluru, India. All the protocols of animal experiments were approved by the Institutional Animal Ethics Committee of SreeVidyanikethan College of Pharmacy, Tirupati, Chittoor Dist., A.P., India (Approval No:SVCP/IAEC/II-08/2019-20) in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Govt. of India, New Delhi. The animals were housed in polypropylene cages and maintained at 24°C±2°C under 12 h light/ dark cycle.

Fig 1: ***Amaranthus roxburghianus leaves***

Nevski

Experimental design: A total of 30 male albino Wistar rats weighing 180–200 gm was selected and allocated to five groups of six rats in each group (n=6).

Group I: Normal control (1% CMC).

Group II: INH + RIF (50 + 50 mg/kg, p.o.)

Group III: INH + RIF (50 +50 mg/kg) + Silymarin (100 mg/kg, b. wt, p.o. 28 days)

Group IV: INH + RIF (50 + 50 mg/kg) + HAAR (200 mg/kg, b. wt, p.o. 28 days)

Group V: INH + RIF (50 +50 mg/kg) + HAAR (400 mg/kg, b. wt, p.o. 28 days).

Animals were killed, 24 h after the last dose. Blood was collected by retro orbital fluxes under ether anesthesia and allowed to clot for 30 min. The serum was separated by centrifugation at 2500 RPM at 30 ℃ for 15 min and used for the estimation of marker enzymes, namely SGOT, SGPT, ALP, TB, TC, TP and TW. The livers were dissected out immediately, washed with ice cold saline and 10% homogenates in 1.15% (w/v) KCl were prepared. The homogenates were centrifuged at 7000 ×g for 10 min at 4 ℃ and the supernatants were used for the assays of SOD, CAT and MDA.

Statistical evaluation:

Data were analyzed by analysis of variance (ANOVA) followed by multiple Comparisons using Dunnett's procedure, to compare all groups against control and Student–Newman–Keul's procedure to compare all the groups pair wise.

RESULTS

Phytochemical screening:

The phytochemical investigation of the hydroalcoholic extract of HAAR was performed by standard methods [14].

Thin layer chromatography

The extracts of leaf of each solvent were subjected to TLC. All spots are colorless in day light but they are colored under UV light. The TLC fingerprint profiling study of present research, justified presence of abundant flavonoids, Phytosterols and Steroids compounds, among which presence of *Amaranthus roxburghianus*. It also showed existence of a number of other separable phytochemicals as well.

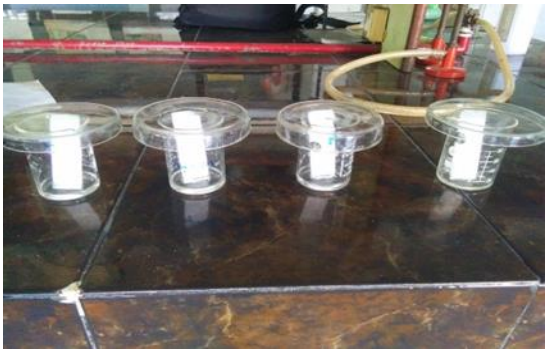


Fig 2: Flow of solvents in TLC medium

SERUM BIOLOGICAL ESTIMATIONS

Effect of Hydroalcoholic extract of A. roxburghianus in different parameters in INH and RIF induced hepatotoxic rats. Increased levels of SGOP, SGOT and ALP levels on HAAR when compared to Anti-tubercular group and mostly equal to standard group. Increased levels of SGPT, SGOT, and ALP in serum of the INH and RIF induced animals certainly indicate liver damage. An increase in the levels of these marker enzymes in serum was due to the leakage of the enzymes from liver as a result of tissue damage. On concurrent treatment with ethanolic extract of A. roxburghianus at dose of 200 and 400 mg/kg respectively, the serum marker enzyme levels were near to normal indicating protection against liver damage.

Values are expressed as mean ± S.E.M.; n=6. Increased levels of TB, TC, TP and TW levels on HAAR when compared to standard silymarin group. The results were compared with the standard silymarin. It is a general perception that, the serum bilirubin levels are elevated in hepatic injury. A marked elevation was observed in serum bilirubin levels of INH and RIF induced rats, whereas total protein (TP) and cholesterol levels in the serum were markedly decreased as silymarin group HAAR levels.

HISTOPATHOLOGY STUDIES

Rats have been successfully employed as models to investigate INH and RIF induced hepatotoxicity. In the present study, INH and RIF was orally administered to induce hepatoxicity in the test animals. Histopathology was undertaken to examine the morphological changes in the hepatic tissue. In the tissue sections, INH exhibited changes in the normal architecture of the parenchyma cells with swelled hepatocytes and loss of the cell boundaries.

Effect of hydroalcoholic extracts of *A. roxburghianus* against anti-tubercular drugs (INH and RIF) induced histopathological changes in normal rat liver. (A) Normal rats showed normal hepatocytes with well-preserved cytoplasm with normal lobular structural design of the liver, (B) anti-tubercular drugs (INH and RIF) induced rat liver, where white arrow indicates necrosis and black arrow indicates inflammation, (C) silymarin (100 mg/kg), (D) and (E) comparison to INH and RIF. The majority of the hepatic lobules preserved the normal architecture with limited hepatic change and hence histopathology score was expressed as "0." Histopathology score data are compiled in the primary reason for the protective effect of INH and RIF is the antioxidant activity of HAAR due to the quenching of free radical and reactive oxygen species.

DISCUSSION

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medica practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulation are used for liver disorders in ethnomedical practices and in traditional system of medicine in India.

Table 1: Phytochemical evaluation of HAAR

S.no	Inference	Test	Observation	Result
1	Carbohydrates	Molisch Test	Violet colour	✓
2	Glycosides	Legals test	Pink to red colour	✓
3	Phytosterols	-	Bluish green colour	✓
4	Tannins	Lead acetate test	No yellowish precipitate	X
5	Amino acids	Ninhydrin test	No Purple colour	X
6	Alkaloids	Dragendroff's test	No yellow precipitates	X
7	Saponins	Froth Formation Test	No froth (foam)	X
8	Steroids	Liebermann's Test	Green colour	✓
9	Flavonoids	Sodium hydroxide test	A canary yellow colour	✓
10	Protein	Million's Test	brick red colour	✓

✓ : indicates presence of phytochemical compound

X : indicates absence phytochemical compound

By the above table the presence of Carbohydrates, Glycosides, Phytosterols, Steroids, Flavonoids and Proteins which are beneficial for pharmacological activities of HAAR.

Table 2: TLC of HAAR

S.NO	Compound	Mobile phase	No. of spots identified	Rf factor
1	Flavonoids	Ethyl acetate: acetic acid: formic acid: Water (5:0.5:0.5:1.35)	3	0.51
2	Saponins	n-butanol: methanol: ammonia (6:1:3)	2	1.03
3	Phytosterols	Chloroform: ethyl acetate (4:6)	3	0.72
4	Steroids	n-heptane: diethyl ether: acetic acid (8.5: 1.5: 0.1)	3	0.47

By the above table represents the presence of Flavonoids, Phytosterols and Steroids which areuseful for pharmacological studies of HAAR.

Table 3: Effect HAAR on SGPT, SGOT and ALP levels

S.no	**Groups**	**SGPT**	**SGOT**	**ALP**
1	Normal control	32.24±1.730029	40.44±2.514558	50.88±2.256768
2	Anti-tubercular dugs	123.52±2.660263	172.62±2.692025	183.28±7.045353
3	Anti TB drugs + Silymarin	51±1.433527	86.48±2.701296	133.5±1.89341
4	Anti TB drugs + 200mg/kg AR	87.32±2.79589	116.34±4.811237	156.92±3.370015
5	Anti TB drugs + 400mg/kg AR	72.16±2.400625	97±12.79219	124.22±17.32879

Values are expressed as mean ± S.E.M.; n=6

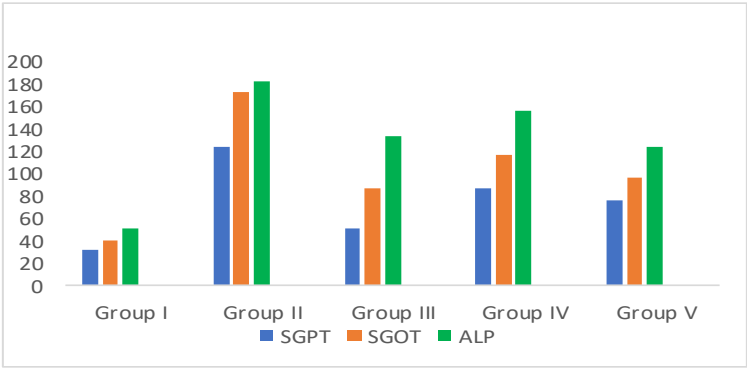


Fig 3: Graphical representation of effect of HAAR ON SGPT, SGOT and ALP

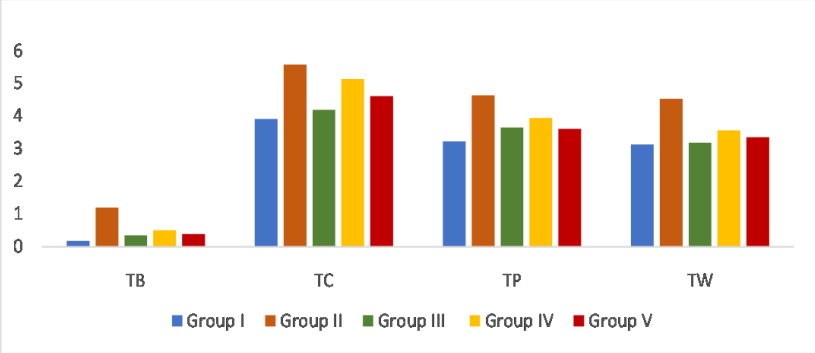


Fig 4: Graphical representation of effect of HAAR on TB, TC, TP and TW

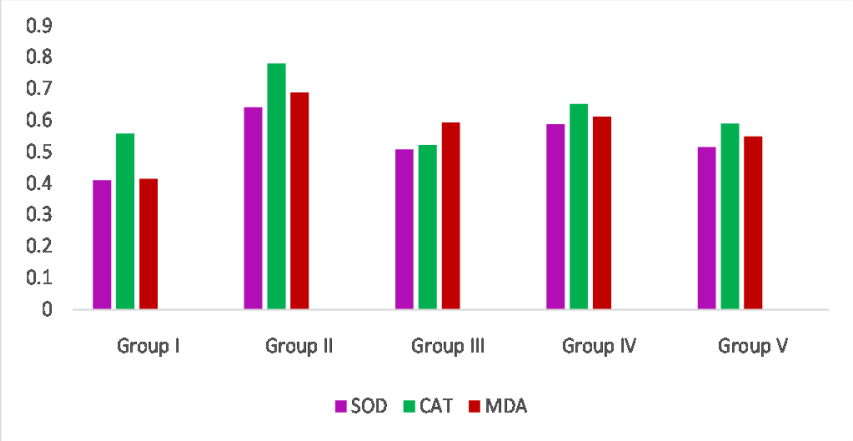


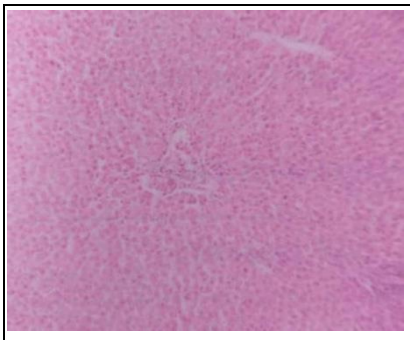
Fig 5: Graphical representation of effect HAAR on SOD, CAT and MDA

Table 4: Effect HAAR on TB, TC, TP and TW

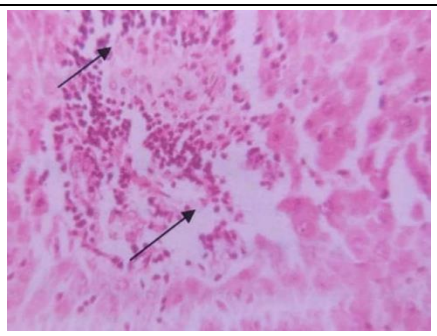
S.no	Groups	Total bilirubin mg/dl	Total Cholesterol	Total Protein
1	Normal control	0.1842±0.042086	3.92±0.12	3.23±0.21
2	Anti-tubercular dugs	1.204±0.112374	5.59±0.19	4.65±0.10
3	Anti TB drugs + Silymarin	0.3542±0.021649	4.20±0.08	3.66±0.15
4	Anti TB drugs + 200mg/kg AR	0.5118±0.131207	5.15±0.144	3.95±0.20
5	Anti TB drugs + 400mg/kg AR	0.3994±0.039564	4.62±0.11	3.62±0.12

Table 5: Effect HAAR on SOD, CAT and MDA

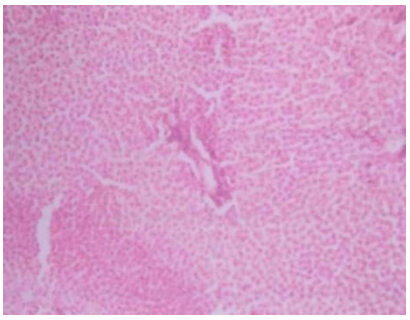
Groups	SOD Units/gm Proteins	CAT mg/proteins	MDA mg/proteins
Normal control	0.412±0.057	0.506±0.035	0.416±0.008
Anti-tubercular dugs	0.643±0.073**	0.782±0.057	0.690±0.112
Anti TB drugs + Silymarin	0.509±0.035**	0.523±0.058**	0.457±0.012**
Anti TB drugs + 200mg/kg AR	0.590±0.028**	0.653±0.112	0.613±0.006**
Anti TB drugs + 400mg/kg AR	0.517±0.032	0.592±0.34**	0.594±0.024**



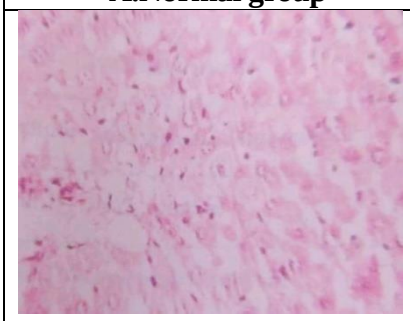
A.Normal group



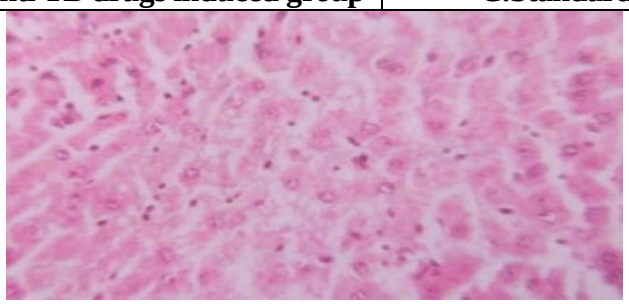
.B.Anti TB drugs induced group



C.Standard group



D. Anti TB + 200mg/kg AR



E. Anti TB + 400mg/kg AR

The present study has demonstrated that AR (200 and 400 mg/kg) exhibited significant dose dependent hepatoprotective activity against liver injury induced by Anti-tubercular drugs. . Both SGOT and SGPT increase due to toxic compounds affecting the integrity of liver cells. Alkaline phosphate is membrane bound glycoprotein enzyme with a high concentration in sinusoids and endothelium. This enzyme reaches liver mainly from the bone. It is excreted into the bile; therefore, its elevation in serum occurs in hepatobiliary diseases. The result of the present study indicates that AR probably stabilizes the hepatic plasma membrane from Anti tubercular drugs induced damage. The SOD, CAT and MDA content had significantly increased ($P < 0.05$ to $P < 0.001$) in HAAR treated groups when compare to standard group whereas antitubercular drugs intoxicated group V had shown significant decrease ($P < 0.001$) in these parameters compared to control group II. Rats have been successfully employed as models to investigate INH and RIF induced hepatotoxicity. In the present study, INH and RIF was orally administered to induce hepatoxicity in the test animals.

Histopathology was undertaken to examine the morphological changes in the hepatic tissue. In the tissue sections, INH Exhibited changes in the normal architecture of the parenchyma cells with swelled hepatocytes and loss of the cell boundaries.

CONCLUSION

The results of this study suggest that the hydroalcoholic extract of *A. roxburghianus* has protective effects against INH and RIF induced hepatic damage in experimental rats. Administration of the extract attenuates the hepatotoxic effects by decreasing MDA production and through an increase of anti-oxidant defenses. Our study demonstrates the health benefits traditionally claimed to this medicinal plant in liver disease. The protective effects shown by HAAR with physical parameters (we liver weight), biochemical parameters (SGOT, SGPT, ALP, TB, TP, TC and TW) and histological parameters recorded with Anti TB drugs induced hepatotoxic rat models clearly depicts that HAAR possessed hepatoprotective activity. The 400mg/kg and 200mg/kg of AR treated groups showed comparable hepatoprotective activity with standard drug silymarin.

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