



***In-vivo* Hepatoprotective and Antioxidant Activities of *Sphaeranthus amaranthoides* Burm.f. Against Anti-Tubercular Drugs Induced Hepatotoxicity in Rats**

M. R. Vinayakamurthi¹, J. Anbu Jeba Sunilson^{1*}, A. V. Anita Gnana Kumari² and U. Aathilakshmi³

¹Department of Siddha Medicine, Tamil University, Thanjavur – 613010, Tamil Nadu, India.

²School of Pharmacy, KPJ Healthcare University College, Kota Seriemas, Nilai 71800, Negeri Sembilan, Malaysia.

³Department of Biotechnology, Periyar Maniammai Institute of Science and Technology, Thanjavur, Tamil Nadu, India.

Authors' contributions

The work was carried out in collaboration among all the authors. Author JAS guided and designed the study. Author MRV performed the hepatoprotective and antioxidant studies. Authors AVAGK and UA helped in the statistical analysis and drafting the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the hepatoprotective and antioxidant activity of *Sphaeranthus amaranthoides* Burm.f. against isoniazid (INH) and rifampicin (RIF) induced hepatotoxicity in rats.

Study Design: Experimental study.

Place and Duration: Research lab, Department of Siddha Medicine, Tamil University, Thanjavur, India, between March 2018 and November 2019.

Methodology: Liver toxicity was induced by antitubercular drugs (Isoniazid; INH+Rifampicin; RIF) at a dose level of 50+100 mg/kg each, p.o for 15 days. Petroleum ether, Chloroform, Methanol,

*Corresponding author: E-mail: anbujsunil@gmail.com;

Aqueous extracts of *Sphaeranthus amaranthoides* Burm.f. (*S. amaranthoides*) (200 and 400 mg/kg bt.wt.) were administered orally once daily for 15 days. The hepatoprotective activity was assessed using various biochemical parameters SGOT, SGPT, ALP, bilirubin, total protein, albumin, total cholesterol, total bilirubin, direct bilirubin and LDH. The antioxidant activities such as the enzymatic activity of superoxide dismutase (SOD), and catalase (CAT), and the level of lipidperoxidation as thiobarbituric acid reactive substance (TBA-RS) were measured in liver homogenates and histological examinations were carried out to assess hepatoprotective activity. For Statistical analysis, the values were subjected to one way analysis of variance (ANOVA) followed by Tukey multiple compare test. Results were considered statistically significant when $P < 0.05$.

Results: The treatment with methanolic extract (400 mg/kg bt.wt.) of *S. amaranthoides* significantly prevented drug-induced increase in serum levels of liver enzymes ($P < 0.001$). The antioxidant activity of a dose of 400 mg/kg of *S. amaranthoides* significantly prevented the decreases in the activity of enzymatic antioxidants (CAT & SOD) ($P < 0.01$ and $P < 0.001$) and inhibited the elevation of lipid peroxidation (TBA-RS) in the liver homogenate. Histopathology of liver tissue showed that *S. amaranthoides* attenuated the hepatocellular necrosis, regeneration and repair of cells toward normal.

Conclusion: The methanolic extract of *S. amaranthoides* showed significant hepatoprotectivity and antioxidant activity against INH + RIF Anti TB drugs.

Keywords: Hepatoprotective; antioxidant; *Sphaeranthus amaranthoides*; antitubercular drugs.

1. INTRODUCTION

Traditional or alternative or complementary systems of medicine are popular not only in developing countries but also in developed countries. Their popularity can be attributed to various historical and cultural reasons. According to the World Health Organization (WHO) [1] estimates around 80% of the population in developing countries relies on plant-derived traditional medicines. Current statistics indicate that the global market for medicinal plants is to the tune of US \$ 62 billion and the demand is growing rapidly. Traditional use of herbal medicines refers to their description in ancient literature and long historical use of those medicines. Their use is well established and widely acknowledged to be safe and effective and may be accepted by national authorities [2]. Indian traditional medicine is based on different systems including Ayurveda, Siddha and Unani with the emerging interest in the world to adopt and study the traditional system and to exploit their potential based on the different health care system, the evaluation of the rich heritage of the traditional medicine is essential [3].

Hepatitis is defined as an inflammation of the liver. It causes an imbalance in the normal function of bile Production, excretion of drugs and hormones, metabolism of fats, proteins, and enzyme activation. According to WHO there are five major types of hepatitis. They are Hepatitis A, B, C, D and E. Hepatotoxicity or liver damage is caused by hepatotoxins, which may source from

chemicals, dietary supplements, pharmaceutical drugs, and medicinal plants. Notably, numerous medicinal plants are used to alleviate illness, particularly in traditional systems of medicine, such as Ayurveda, Siddha, and Traditional Chinese medicine. These systems of medicine have been implemented for centuries for treating various ailments. Some medicinal plants serve as hepatoprotectors against liver damage, while others induce hepatotoxicity.

Hepatitis B virus (HBV) infection continues to remain a significant global health problem. Estimates of the WHO suggest that more than 2 billion people worldwide have been infected with HBV. Of these, approximately 240 million individuals have chronic (long-term) liver infections and at risk of serious illness and death, mainly from liver cirrhosis and hepatocellular carcinoma (HCC). More than 780 000 people die every year due to the acute or chronic consequences of hepatitis B. India has an approximately HBV carrier rate of 3.0% with a high prevalence rate in the tribal population. With a population of more than 1.25 billion, India has more than 37 million HBV carriers and contributes a large proportion of this HBV burden. While horizontal transmission in childhood appears to be a major route of transmission, the role of vertical transmission is probably underestimated. Blood transfusion and unsafe therapeutic injections continue to be important modes of transmission of HBV. There is a need for large field studies to better understand HBV epidemiology and identify high

prevalence areas, and public health measures to prevent disease transmission and decrease the burden of the disease [4].

S. amaranthoides is a small procumbent herb with spreading branches found in a semi-aquatic environment. It belongs to the family, Asteraceae. In Tamil, it is known as Sivakaranthai. It is used as an energizer in Siddha preparation. This plant was known for the treatment of eczema, blood disorders, stomach worms, filaria, fever, skin diseases, anti-helminths and jaundice [5]. According to the Siddha system and literature review, the plant *S. amaranthoides* have many medicinal uses [6]. Based on the potential benefits of the plant and in scientific view the current research focuses on the evaluation of hepatoprotective and antioxidant activity of *S. Amaranthoides*.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant

The whole plant of *S. amaranthoides* was collected from Reddiyar Patti Village, Tirunelveli District, Tamilnadu in the month of December 2017. The plant was carefully examined and authenticated by Prof. P. Jayaraman, M.Sc., Ph.D., Director, Plant Anatomy Research Center, Chennai, Tamilnadu, India. A voucher specimen has been deposited for future reference (voucher specimen number 21A3).

2.2 Preparation of Plant Extract

Coarsely powdered whole plant of *S. amaranthoides* (2 kg) were extracted with 5 L of solvents such as Petroleum ether (PE), Chloroform (CE), Methanol (ME), and Aqueous (AE) by continuous hot percolation method using Soxhlet Apparatus. At the end of each extraction, they were filtered through filter paper and concentrated under vacuum using a rotary vacuum evaporator. These extracts were used for the Pharmacological studies [7].

2.3 Animals

Wistar strain albino rats of either sex weighing 150-200 g were used for this study. Animals were housed in cages at an ambient temperature of $25 \pm 2^\circ\text{C}$ and 45 -55% relative humidity with 12 hours light / dark cycle. They will have free access to standard pellet chow (Brook Bond, Lipton India) and water *ad libitum*. Animals were divided into 11 groups of 6 animals each.

2.4 Acute Toxicity

Acute toxicity study “up and down procedure” was carried out as per the guidelines set by Organization for the Economic Co-operation and Development (OECD) [8]. If animals die at a particular dose, the lower dose was given to the next animal and if the animal survives at a particular dose, the next higher dose was given for remaining animals. The maximum upper limit dose of 2000 mg/kg of *S. amaranthoides* extracts was administered to mice. Animals were observed individually after doing. Observation included mortality and clinical signs, such as changes in skin fur, eyes, and mucous membranes. The gross behavior as body position, locomotion, rearing, tremors, the gait were observed. The effect of *S. amaranthoides* on passivity, grip strength, pain response, stereotype, vocalization, righting reflex, body weight, and water intake were assessed.

2.5 Grouping of Animals

Wistar strain albino rats were divided into 11 groups of 6 animals each. Normal control group (Group I) were given only vehicle (5 % acacia solution) without hepatotoxicity, whereas animals from the model control group (Group II) were only given Isoniazid at a dose of 50 mg/kg b.wt. + Rifampicin at a dose of 100 mg/kg b.wt. (INH + RIF) without any treatment. Animals from Group III to X were given Pet ether (PE), Chloroform (CE), Methanol (ME), and Aqueous extracts (AE) of *S. amaranthoides* (200 mg / 400 mg /kg p.o) for 15 days. Group XI was given standard drug silymarin (50 mg/kg p.o).

2.6 Hepatotoxins

Hepatic damage was induced by administration of Isoniazid (50 mg/kg) and Rifampicin (100 mg/kg) orally, once daily induced hepatic injury. Drugs were given as suspension orally 45 minutes before standard and test drug treatment. The animals were killed on the 15th day after the dose of Isoniazid – Rifampicin administration. Rats will have free access to food and drinking water during the study [9].

2.7 Assessment of Hepatoprotective Activity

The serum was used for estimating the biochemical parameters viz., glutamic

oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin (BL), total cholesterol (TC), lactate dehydrogenase (LDH), albumin, total protein (TP), total bilirubin (TB) and direct bilirubin (DB) by using standard assay kit from Span diagnostics, Surat, India. [10].

2.8 Assessment of Antioxidant Parameters

Hepatic tissues of rats were homogenized (10%) in phosphate buffer (pH 7.4) with a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged at 12000 rpm for 20 min at 4°C to obtain post mitochondrial supernatant (PMS) and it was used for the estimation of the activity of catalase (CAT), superoxide dismutase (SOD), and lipid peroxidation as thiobarbituric acid reactive substance (TBA-RS) in the PMS of liver [10].

2.9 Histopathology Studies

At the end of the treatment, rats were sacrificed by cervical decapitation. The liver was excised, washed in physiological saline to remove the blood clot and other tissue materials, and a portion of liver was fixed in 10% formalin saline. The section was prepared and stained with hematoxylin and eosin for the histological investigations

2.10 Statistical Analysis

A result of biochemical estimation has been expressed as mean ± Standard Error of Mean (SEM). The values were subjected to One Way Analysis of Variance (ANOVA) using Graph Prism version 3.0. The variance in a set of data has been estimated by Tukey multiple compare test. The values of P<0.05 were considered statistically significant, P<0.01 highly significant and P<0.001 considered very highly significant.

3. RESULTS

3.1 Acute Toxicity Study

Table 1 represented the results of acute toxicity studies in albino mice administered with various extracts of plants by the oral route. The test extracts were suspended in 0.5% carboxyl methylcellulose (CMC) and administered orally. No acute mortality was observed even at 2000 mg/kg for various test extracts of *S. amaranthoides*. All the animals were found to be normal and there were no gross behavioral changes till the end of the observation period (14 days). From these results, LD₅₀ or the maximum tolerated dose was found to be 2000 mg/kg. From this, 1/5th and 1/10th of the maximum tolerated dose (200,400 mg/kg) was selected for the screening of hepatoprotective activity.

3.2 Effect of *S. Amaranthoides* Extract on Liver Marker Levels

The results of hepatoprotective effects of methanolic, chloroform, petroleum ether and aqueous extracts of *S. amaranthoides* on INH +RIF intoxicated rats are shown in Table 2. Administration of INH+RIF at a dose of 50 + 100 mg/kg body weight p.o. each significantly (P<0.001) elevated SGPT, SGOT, ALP, LDH, TB and DB while TC,TP and Albumin were significantly decreased (P<0.001) when compared to control group treatment of methanolic extract of *S. amaranthoides* at a dose of 200 and 400 mg/kg, 45 minutes prior to INH + RIF administration significantly reversed the elevation of biochemical enzymes such as SGOT, SGPT, ALP, LDH and bilirubin. The protection was better on dose 400 mg/kg and a significant increase (P<0.001) was observed in the levels of TP, TC and albumin in the serum, against the hepatotoxic control group. Administration of silymarin significantly reversed (P<0.001) the altered liver markers levels when compared to hepatotoxic group.

Table 1. Acute toxicity study of various extracts of *S. amaranthoides*

S. no.	Extract	LD ₅₀ or MTD (mg/kg)	ED ₅₀ or 1/5 th of LD ₅₀ (mg/kg)	ED ₅₀ or 1/10 th of LD ₅₀ (mg/kg)
1.	Pet. Ether extract (PE)	2000	400	200
2.	Chloroform extract (CE)	2000	400	200
3.	Methanolic extract (ME)	2000	400	200
4.	Aqueous extract (AE)	2000	400	200

MTD - Maximum Tolerated Dose; LD₅₀ - Lethal dose producing lethal effect in 50% population; ED₅₀ - Effective dose producing pharmacological effect in 50% population

Table 2. Effects of various extracts of *S. amaranthoides* on biochemical parameters of anti-TB DRUG intoxicated rats

Treatment groups (mg/kg)	SGOT U/l	SGPT U/l	ALP U/l	LDH U/l	TC mg/dl	TP g/l	Albumin g/l	Total bilirubin mg/dl	Direct bilirubin mg/dl
Control	72.5± 0.57	46.8± 0.67	322.00 ±2.30	351.25 ± 3.061	78.1± .576	7.66± 0.11	3.21± 0.11	0.33± 0.09	0.16 ± 0.007
AT Drugs	462.78 [#] ± 2.99	326.00 [#] ± 3.535	450.65 [#] ± 2.171	591.42 [#] ± 2.418	28.6 [#] ± 0.194	4.44 [#] ± 0.05	1.84 [#] ± 0.24	2.51 [#] ± 0.02	1.43 [#] ± 0.033
PE 200 + AT	371.4 ± 2.66	300.46 ± 2.91	411.53± 3.48	563.24± 3.391	34.86± 1.32	4.67± 0.52	2.08 ± 0.032	2.19 ± 0.018	1.29 ± 0.098
PE 400 + AT	383.59± 2.89	298.65 ± 3.125	420.96 ± 4.593	581.62± 3.638	34.07± 1.611	4.59± 0.84	1.98 ± 0.068	2.08 ± 0.033	1.19 ± 0.041
CE 200 + AT	423.81±7.13	312.93±4.18	423.51± 4.36	562.68 ± 3.08	31.63± 2.91	4.36± 0.41	1.89 ± 0.023	1.86* ± 0.062	0.87* ± 0.017
CE 400 + AT	361.62± 2.14	299.78± 3.46	401.08 ± 3.991	542.92± 4.89	38.62 ± 0.36	5.18 ± 0.04	2.12 ± 0.086	2.03 ± 0.018	1.13 ± 0.029
ME 200 + AT	358.72 ± 5.51	278.63 ± 3.11	412.82± 6.328	498.52± 9.694	36.41± 1.39	5.23 ± 1.32	2.61 ^{**} ± 0.072	2.37 ± 0.098	1.38 ± 0.051
ME 400 + AT	74.76 ^{***} ± 4.24	48.44 ^{***} ± 3.19	322.39 ^{***} ± 9.10	351.9 ^{***} ± 5.09	73.03 ^{***} ± 4.611	7.54 ^{***} ± 1.81	3.09 ^{***} ± 0.07	0.35 ^{***} ± 0.013	0.18 ^{***} ± 0.01
AE 200 + AT	298.36 [*] ± 4.42	262.53 ± 3.321	417.58±5.756	521.52 ± 7.833	33.25 ± 2.99	4.98± 0.5	1.93 ± 0.061	1.61 [*] ± 0.047	0.78 [*] ± 0.019
AE 400 + AT	94.8 ^{**} ± 5.07	60.5 ^{**} ± 3.11	336.00 ^{***} ± 3.307	372.00 ^{**} ± 3.602	62.9 ^{**} ± 2.77	5.25 ± 0.30	2.04±0.151	0.58 ^{**} ± 0.015	0.33 [*] ± 0.042
S 50 + AT	73.31 ^{***} ± 1.95	48.01 ^{***} ± 2.72	322.84 ^{***} ± 2.31	352.61 ^{***} ± 0.564	76.85 ^{***} ± 1.39	6.99 ^{**} ± 0.26	2.28 ^{***} ± 0.04	0.29 ^{***} ± 0.003	0.16 ^{***} ± 0.004

Values are expressed as mean ± SEM of 6 rats in each group; P value: * <0.05, ** <0.01, *** <0.001 compare with group II (INH + RIF); P value: # <0.001 compare with respective normal control group I

3.3 Effect of *S. amaranthoides* Extract on Anti-oxidant Parameters

Activities of hepatic SOD, CAT, and TBA-RS are presented in Table 3. The enzyme activity of SOD and CAT had significantly ($P < 0.001$) increased while the level of TBA-RS were significantly ($P < 0.001$) decreased in *S. amaranthoides* methanolic extract treated group. When compare to group II whereas anti tubercular drugs intoxicated group II had shown significant decrease ($P < 0.001$). There is an increasing TBA-RS was observed in anti TB drug intoxicated rats ($P < 0.001$). Treatment with *S. amaranthoides* at the doses of 200 and 400 mg/kg significantly prevented this elevation in levels in the enzymatic antioxidant were ($P < 0.01$ and $P < 0.001$) respectively. In different doses level of *S. amaranthoides* 400 mg/kg has shown maximum protection which was almost comparable to those of the normal control and silymarin.

3.4 Histopathological Observation

Microscopic examination on normal liver section shows normal parenchymal cells. Abundant

eosinophilic cytoplasm, consisting of cells with normal vesicular nuclei with nucleoli and Mucosal glands are seen compactly arranged (Fig. 1(a)). The degeneration and necrosis of liver cells, presence of pycnotic nuclei, granular cytoplasm and increase in inter cellular spaces with inflammatory collections, hepatocellular necrosis was found (Fig. 1 (b)). In rats group treated with *S. amaranthoides* extracts in two dose level (200 and 400 mg/kg b.wt.) (Fig. 1(c-f)) shows marked changes at the granular cytoplasm, periphery and decrease in intercellular spaces as compared to AT drug intoxicated control rats. Liver sections show minimal degenerative changes of hepatocytes with minimal inflammation. The treatment of methanolic extracts with 400mg/kg showed that there is a significant reduction in tissue damage, hepatocellular necrosis, regeneration of new cell and repair cell to normal. Group XI treated with silymarin 50 mg/kg bt.wt. as reference drug (Fig. 1 (g)) shows intact parenchymal cells, abundant eosinophilic cytoplasm, regeneration of new cells which was well compare with normal and methanolic extract 400 mg/kg b.wt. treated groups. Histopathological study supports the biochemical findings.

Table 3. The effect of various extract of *S. amaranthoides* and silymarin on the enzymic antioxidant of anti-tubercular drugs intoxicated rats

Treatment groups (mg/kg)	Catalase μ moles of H_2O_2 utilized /min/mg of protein	SOD NBT reduction / min / mg protein	TBA-RS mmoles/mg tissue
Control	15.21 \pm 0.128	2.982 \pm 0.361	0.521 \pm 0.063
AT Drugs	6.855 [#] \pm 0.124	0.826 [#] \pm 0.011	3.028 [#] \pm 0.072
Pet ether 200 + AT Drugs	8.621 \pm 0.194	0.897 \pm 0.043	2.711 \pm 0.325
Pet ether 400 + AT Drugs	8.629 \pm 0.211	0.854 \pm 0.098	2.341 \pm 0.096
Chloroform 200 + AT Drugs	8.618 \pm 0.738	1.382 \pm 0.051	2.589 \pm 0.119
Chloroform 400 + AT Drugs	9.555 [^] \pm 0.196	1.563 \pm 0.088	2.367 \pm 0.825
Methanolic 200 + AT Drugs	12.978 ^{***8} \pm 0.862	3.505 ^{***} \pm 0.129	0.788 ^{***} \pm 0.043
Methanolic 400 + AT Drugs	14.18 ^{***} \pm 0.132	3.929 ^{***} \pm 0.174	0.672 ^{***} \pm 0.078
Aqueous 200 + AT Drugs	9.83 [^] \pm 0.158	0.816 \pm 1.26	2.241 \pm 0.173
Aqueous 400 + AT Drugs	10.183 ^{**} \pm 0.798	1.738 \pm 1.74	1.973 [^] \pm 0.136
Silymarin 50 + AT Drugs	13.185 ^{***} \pm 0.126	3.415 ^{***} \pm 0.196	0.563 ^{***} \pm 0.019

Values are expressed as mean \pm SEM of 6 rats in each group; P value: [^] <0.05 , ^{**} <0.01 , ^{***} <0.001 compare with group II (INH + RIF); P value: [#] <0.001 compare with respective normal control group I

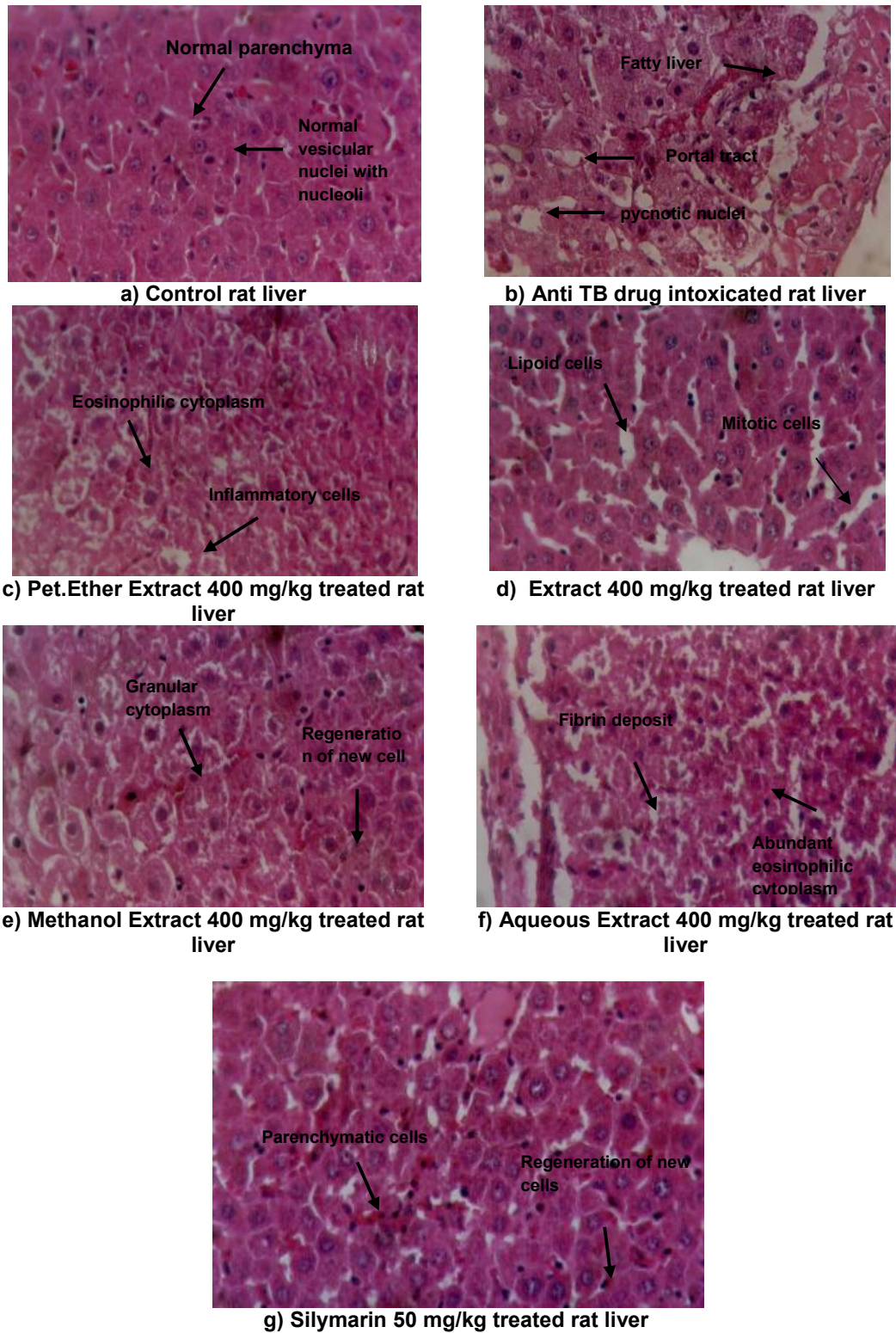


Fig. 1. Effect of various extracts of *S. amaranthoides* against AT drugs induced histopathological changes in rat liver

4. DISCUSSION

In the present study, *S. amaranthoides* was evaluated for hepatoprotective and antioxidant activity using antitubercular drugs (INH + RIF) induced liver toxicity in rats. Drug-induced liver toxicity is a probably severe adverse effect of the currently used antitubercular chemotherapeutic regimens containing isoniazid and rifampicin.

The combination of INH + RIF anti-tubercular drugs-induced hepatotoxicity showed mainly as hepatocellular steatosis and centrilobular necrosis may be associated with cholestasis, and it has been recommended that toxic isoniazid metabolites bind covalently to cell macromolecules in both animal and human case studies [11]. The conversion of mono acetyl hydrazine, a metabolite of isoniazid, to a toxic metabolite through cytochrome P450 prompts hepatotoxicity. Rifampicin induces cytochrome P450 enzyme causing increased production of toxic metabolites from acetyl hydrazine (AcHz). Rifampicin can increase the metabolism of Isoniazid to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half-life of AcHz is shortened by Rifampicin and AcHz is immediately converted to its active metabolites by improving the oxidative elimination rate of AcHz, which is similar to the higher rate of liver necrosis caused by INH and RIF in combination [12]. In addition to these mechanisms; oxidative stress induced hepatic injury is one of the important mechanisms in hepatotoxicity produced by antitubercular drugs [13].

On treatment with methanolic extract of *S. Amaranthoides* at dose of 400 mg/kg the serum marker enzyme levels were near to normal indicating protection against liver damage (Table 2). The results were compared with the control group the marked elevation was observed in SGOT, SGPT, ALP, LDH and bilirubin levels of INH+RIF induced rats, whereas Total protein (TP), Total cholesterol (TC) and albumin levels in the serum were markedly decreased. The body has an effective defense mechanism to prevent and neutralize the free radical induced damage. This is proficient by a set of endogenous antioxidant enzymes such as SOD, and catalase. Suppression of the antioxidant system in anti-tubercular drugs intoxicated rats has been reported earlier [14]. The significantly reduction in TBA-RS shows hepatic damage in the rats administered with antitubercular drugs but on treatment with 200 and 400 mg/kg of *S. Amaranthoides* extracts showed significant

increase in the level of these enzymes due to the ability of the phytoconstituents scavenge reactive oxygen species. Increase in the level of lipid peroxides in liver reflected the hepatocellular damage in Anti TB drug treated group. The raise in lipid peroxidase leading to oxidative stress-induced cell death [15]. In the present study which indicates the antioxidant activity of the *S. amaranthoides* shows a reverse in antioxidant after the treatment of *S. amaranthoides* 400mg/kg b.wt. methanolic extract treated rat livers which will compared with control group and silymarin group. The histopathological findings further confirms the hepatoprotective activity of the *S. amaranthoides* plant extract. The photochemical screening of *S. amaranthoides* reveals the presence of flavanoides, glycosides and steroids are the major constituents, hence the hepatoprotection of *S. amaranthoides* may be due to its antioxidant activity [16].

5. CONCLUSION

The methanolic extract of *S. amaranthoides* has shown dose-dependent activity against Isoniazid + Rifampicin induced hepatic damage in experimental rats. The hepatoprotective effect of the *S. amaranthoides* was further validated by the histopathological examinations. Further investigation of these promising protective effects of *S. amaranthoides* against antitubercular drug induced hepatic injury may have a significant impact on developing clinically possible strategies to treat patients with hepatotoxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimentation on animals was approved by the Institutional ethical committee (Ref no: 791/03/b/CPSC) under the regulation of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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