Original Research Article

HEPATOPROTECTIVE ACTIVITY OF *PONGAMIA PINNATA* LEAVES ON ANTITUBERCULAR DRUGS (ISONIAZID & RIFAMPIN) INDUCED HEPATOTOXICITY IN RATS.

ABSTRACT:

Hepatoprotective effect of ethanolic extract of *Pongamia Pinnata* on antitubercular drugs (isoniazid and rifampin) induced hepatotoxicity in rats.

Methods: The experiment used five groups of male wistar rats, each with six individuals. The two control groups were given gum acacia and a mixture of isoniazid and rifampin. The two test groups received 200 and 400 mg/kg of an ethanolic extract of the leaves of *Pongamia Pinnata*, respectively. The fifth group was given silymarin (50mg/kg, orally). The concentrations of serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), tissue Malondialdehyde (MDA) & thiols were calculated. One-way ANOVA was used for statistical examination, monitored through Tukey's test.

Results: When rats were given a mixture of antitubercular medications and a high dosage (400 mg/kg) of ethanolic extract of *Pongamia Pinnata*, blood enzyme levels were lower than when they were given antitubercular drugs alone. The coadministration of a high dose of *Pongamia Pinnata* extract with antitubercular medicines reduced MDA levels and elevated thiol levels considerably ($p < 0.05$). These biochemical marker levels, however, were not adjusted.

Conclusion: In rats, *Pongamia Pinnata* encompasses a partial protective effect against the hepatotoxicity caused by antitubercular medicines at high doses.

Keywords: *Pongamia Pinnata, isoniazid, rifampin, hepatoprotective.*

1. INTRODUCTION:

Hepatic damage could be a common side effect of first-line antitubercular medicines such pyrazinamide, isoniazid, and rifampin [1]. This has an impact on patient compliance, which may result in therapy termination and resistance development.

The plant *Pongamia Pinnata* is also known as karanja. Various components of the plant are used as a crude medication to cure skin illnesses, tumours, piles, itches, rheumatic joint sores, diarrhoea, and ulcers, among other ailments [2]. It's been employed in the Ayurvedic and Unani systems of medicine as an anti-oxidant [3], anti-plasmodial [4], anti-diabetic [5], anti-diarrheal [6], anti-hyperammonemic [7], anti-nociceptive & antipyretic [8], anti-ulcer [9], anti-inflammatory [10] and other therapeutic characteristics.

Pongamia Pinnata has been shown to be high in phenolics [11] and flavonoids [12]. Polyphenolics are antioxidants that can be found in nature [13]. In addition, it has a long history of use in the treatment of liver problems within the existing literature, however, no scientific data on its hepatoprotective efficacy has been published. As a consequence, the present-day study was accompanied to conclude the hepatoprotective impression of *Pongamia Pinnata* leaves.

2. INGREDIENTS & PROCEDURES

2.1. METHOD:

The Animal Research Committee at Raghavendra Institute of Pharmaceutical Education and Research (RIPER) in Ananthapuram, Andhra Pradesh, approved the experimental protocols. All of the animals were cared for in accordance with the CPCSEA's guidelines.

2.2. CHEMICALS:

Isoniazid & rifampin were acquired in pure form from Micro Labs in India. Alkaline phosphatase (ALP), Alanine Transaminase (ALT) & Aspartate Aminotransferase (AST) were all measured using diagnostic kits as of Ranbaxy in New Delhi. All of the medicines, compounds, & reagents utilized in the biological estimate remained given by Sigma-Aldrich in the United States.

2.3. ANIMALS:

Wistar albino rats weighing 200–250 gr. be there procured commencing RIPER's animal house and maintained in an air-conditioned environment by 12 hr. light/dark cycles, a continuous temperature of 22°c & a relative humidity of 65–70%. The animal ethics committee **(Approval No-07/IAEC/2011)** gave its seal of approval to all experimental protocols.

2.4. PLANT COLLECTION:

Leaves of *Pongamia Pinnata* were taken from its natural habitat on the RIPER campus in the

Ananthapuram district of Andhra Pradesh in December 2020. The plant was verified by S.V. University's Department of Botany in Tirupati, Andhra Pradesh. To avoid volatile oil deterioration, the leaves were cleaned & dehydrated in the shadow. The leaves were dried out in hot air woven for three days by 55°C and then aimed at four days by 40^{O}C

2.5. PREPARE THE PLANT EXRACT:

The dried and powdered leaves (200 gm each) were separately soaked in 1000 ml of distilled water.The mixture is stirred periodically using sterile glass rod up to 72 hours. Each solvent extract was collected separately and dried using rotary vacuum evaporator followed by lyophilizer and stored in dessicator until further use [14].

2.6. STUDY DESIGN:

Five groups of rats were used in the experiment. Isoniazid (INH) 50 mg/kg Intra Peritoneally (IP), rifampin (RI) 100 mg/kg i.p. body weight of rats were utilised as hepatotoxic medicines in each group of six rats [15, 16]. Silymarin (hepatoprotective) was given at a dosage of 50 mg/kg p.o. during this study [17]. *Pongamia Pinnata* ethanolic extract was given orally in dosages of 200 mg/kg & 400 mg/kg. All of the medicines were given to the rats once on a daily basis for 21 days in an exceedingly 2% gum acacia suspension [15].

Group I: Normal Control (was administered 2% Gum acacia, 10 ml/kg p. o. and 10 ml/kg i. p.)
Group II: Toxic Control (was given a combination of (INH + Rifampin i.p.))

Group III: Tratment group (was treated with (INH + Rifampin i.p.) along with extract of Pongamia Pinnata 200mg/kg. orally)

Group IV: Tratment group (was treated with (INH + Rifampin i.p.) along with extract of Pongamia Pinnata 400mg/kg. orally)

Group V: Standard group (was given (INH + Rifampin i.p.) along with silymarin orally)

Each rat was sacrificed using an excess of ketamine about 24 hours after the previous dosage of medication was administered. Samples of blood were taken through puncture of the heart to determine serum hepatic enzyme levels such as aspartate aminotransferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP). The liver was torn out, washed in cold 0.9 percent saline, floated in phosphate buffer, weighed, homogenised, and therefore the

thiols and MDA in hepatic tissue were calculated [18,19].

2.7. Statistical analysis:

Tukey's test was applied after a one-way ANOVA. The data was accessible as a mean standard deviation. The consequence level was fixed at $P < 0.05$.

3. RESULTS

Hepatotoxicity by antitubercular drugs resulted in significantly ($P<0.05$) elevated levels of serum AST, ALT and ALP levels as compared to control. The rise in levels of serum enzymes was lower in rats treated with a combination of antitubercular drugs and high dose (400 mg/kg) of ethanolic extract of *Pongamia Pinnata* compared to those administered hepatotoxic drugs alone **(Table 1).** The plant extract at a lower dose (200 mg/kg) was unable to prevent the rise in serum enzyme levels caused by antitubercular drugs. The standard drug, silymarin, prevented a rise in serum hepatic enzyme levels when co administered with antitubercular drugs.

Table 1: The effect of antitubercular medications given in combination with an ethanolic extract of Pongamia Pinnata on blood AST, ALT, and ALP levels in rats.

GROUPS (n=6)	DRUS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I	Gum acacia	158.46±31.22	64.56±5.46	263.46±16.76
II	INH + RIF (Toxic Control)	348.16±29.26*	79.46±6.76*	348.48±20.53*
III	INH + RIF + PP (Low dose)	323.46±14.98	73.45±9.54	332.26±8.86
IV	INH + RIF + PP (High dose)	292.62±19.46**	65.59±5.64**	315.25±19.62**
V	INH + RIF + Silymarin (Std.)	146.45±11.33$^{**, \Delta, \#}$	58.56±4.65$^{**, \Delta}$	257.76±21.76$^{**, \Delta, \#}$

n= number of rats in each group

*p < 0.05 vs Group I; ** p < 0.05 vs Group II; Δ p < 0.05 vs Group III

#p< 0.05 vs Group IV

Treatment with antitubercular medicines resulted in a substantial (p <0.05) increase in hepatic MDA but a substantial reduction in thiol levels when paralleled to the control group. Antitubercular medicines combined with a high dose of *Pongamia Pinnata* extract significantly (p<0.05) reduced MDA levels while increasing thiol levels in rats **(Table 2).** The plant extract at a lower dose (200 mg/kg) had no effect on liver MDA and thiol levels.

Table 2: Effect of antitubercular medicines given together with an ethanolic extract of *Pongamia Pinnata* on hepatic MDA and thiol levels in rats

GROUP (n = 6)	DRUG	MDA (nmol/mg)	THIOLS (nmol/mg)
I	2% Gum acacia	86.23±9.12	4.68±0.15
II	INH + Rifampin	157.48±11.24*	2.46±0.11*
III	INH + Rifampin + PP(200mg)	146.23±12.89	2.65±0.23
IV	INH + Rifampin + PP(400mg)	111.68±9.42**	2.46±0.24**
V	INH + Rifampin + Silymarin	93.69±11.12**, Δ	3.76±0.12**, Δ, #

n= number of rats in each group

*p < 0.05 vs Group I; **p < 0.05 vs Group 2; Δp < 0.05 vs Group 3;

#p< 0.05 vs Group IV

4. DISCUSSION:

The present study looked into the efficacy of ethanolic extracts of *Pongamia Pinnata* leaves to protect against hepatotoxicity caused by isoniazid and rifampin. The liver could be a significant metabolic organ that is impacted by many chemicals and toxins, and liver damage caused by diverse hepatotoxins have long been recognised as a serious toxicological issue [20].

The production of free radicals by antitubercular medicines causes hepatocellular damage [15]. Lipid peroxidation is caused by free radicals, which results in cell membrane breakdown. Damage to the membrane allows enzymes to leak out, resulting in higher amounts of enzymes within the blood. MDA is formed as a result of lipid peroxidation. As a result of the production of free radicals, antioxidants such as hepatic thiols are consumed, reducing their levels.

Silymarin is a polyphenolic antioxidant found in the Silybum marianum plant. It's been utilised as an immunostimulant, hepatoprotectant, and nutritional supplement for centuries. Silymarin also functions as a chemopreventive and anti-cancer agent [21]. The presence of flavonolignans primary is responsible for its hepatoprotective properties [22]. It has high free radical scavenging properties, reduces lipid peroxidation, and promotes hepatocyte regeneration. Furthermore, silymarin inhibits the 5-lipoxygenase pathway and has membrane-stabilizing effects. All of these components work together to give it hepatoprotective properties [23].

The phenolics [11] and flavonoids [12] in the leaves extract of *Pongamia Pinnata* have been discovered using various solvents. Polyphenols found in *Pongamia Pinnata* have been shown to have free radical scavenging properties in studies [6]. As demonstrated by a decrease in hepatic MDA and an increase in thiols in rats given the extract, this could have resulted in a reduction in oxidative stress.

5. CONCLUSION:

In large doses, *Pongamia pinnata* protected against antitubercular drug-induced hepatotoxicity, however the protection was only limited because it did not normalise serum enzymes, liver MDA, or thiol levels.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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