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Comparative study on hepatoprotective activity of Pongamia pinnata (PP) & Annona squamosa (AS) leaf extracts against anti-tubercular drugs (isoniazid & rifampin) induced hepatotoxicity in rats

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***Abstract*---**Objectives: - The goal of this study is to compare the effects of Pongamia Pinnata and Annona Squamosa on anti-tubercular

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medicines-induced hepatotoxicity in rats. Materials and Procedures: - In rats, hepatotoxicity was caused by administering a suspension of isoniazid and rifampin orally for 21 days. Pongamia Pinnata and Annona Squamosa, as well as anti-tubercular medicines, were given to the treatment groups. Biochemical & histological criteria were used to measure liver destruction. Results: - The use of Pongamia Pinnata and Annona Squamosa in combination with anti-tubercular medicines dramatically reduced Serum Glutamate Pyruvic Transaminase (SGPT), Serum Glutamate Oxaloacetic Transaminase (SGOT)& tissue malondialdehyde (MDA) levels. Inflammation, degeneration, and necrotic alterations in hepatocytes were reduced. Pongamia Pinnata also reduced a drop in blood Superoxide Dismutase (SOD) when compared to a control group getting only anti-tubercular medicines. Pongamia Pinnata, on the other hand, had no statistically significant effects when compared to Annona Squamosa and silymarin. Conclusion: - Annona Squamosa was found to be an effective hepatoprotective agent in rats, as it considerably reduced the hepatotoxic damage caused by anti-tubercular medicines. However, when the effects of Pongamia Pinnata & Annona Squamosa or silymarin were compared, there was no statistically significant difference. As a result, it is only as good and effective as Pongamia Pinnata, contrary to popular assumption.

***Keywords*---**Pongamia pinnata, Annona squamosa, isoniazide, rifampin, silymarin, hepatoprotective.

Introduction

Hepatotoxicity caused by anti-tubercular medicines is a potential side effect of current anti-tuberculosis regimens that affects roughly 9% of individuals with active tuberculosis (TB) [1, 2]. The most successful anti-tuberculosis therapy (standard therapy) is an eight-week course of isoniazid (INH), rifampin (RIF), and pyrazinamide (PZA), followed by another four to seven months of INH and RIF [3]. However, there is substantial evidence of these standard medications toxicity in humans [4, 5, 6], with hepatotoxicity being the most serious side effect [7]. Hepatotoxicity rates have been shown to be substantially greater in underdeveloped nations (8–30%) than in advanced countries (2–3%) with a same dosing schedule [8]. Hepatotoxicity make difficulties the management of 5–10% of individuals with dynamic tuberculosis. [9]. Hepatotoxicity caused by Isoniazid is considered idiosyncratic since it is most likely caused by toxic metabolites rather than hypersensitivity or allergic reaction. [10, 11]. Acetylation through the hepatic enzyme N-acetyl transferase 2 is the most common metabolic pathway of INH metabolism (NAT 2) [12]. It's acetylated to form acetylisoniazid, which is then hydrolyzed to form acetylhydrazine and isonicotinic acid. Acetylhydrazine is either hydrolyzed to produce hydrazine or acetylated to produce diacetyhydrazine[11,13]. Desacetylation to desacetylrifampicin is the main metabolic pathway for rifampicin, and separate hydrolysis yields 3 – formylrifampicin [14,15]. The pathogenesis of INH and RIP-induced damage may involve oxidative stress in the liver mitochondria, which is linked to changes in

mitochondrial permeability and increased hepatocyte apoptosis. As a result, mitochondrial redox changes have been proposed as crucial events in INH- RIF induced apoptotic liver cell injury.

Pongamia Pinnata (Family: Leguminosae) is a semi-evergreen tree of modest stature, has a small bole, a spreading crown, and bark that is greyish green or brown that grows up to 18 m or higher. Imparipinnate, alternate leaves with 5-7 oval & opposite leaflets. In Hindi, this tree is familiar as Karanja, while in English, it is known as Indian Beech or Derris indica, & in Kannada, it is known as Hongae. Pongamia Pinnata can be found growing along the banks of rivers & streams in India, as well as being planted as an avenue tree in gardens. The leaves of the Pongamia Pinnata have been reused in Ayurvedic medication as an anthelmintic, laxative and digestive as well as to treat piles, wounds, relieve rheumatic pains and clean ulcers in gonorrhoea & scrofulous development [16]. Pongamia Pinnata L. is used to treat hypertension, skin problems, rheumatic arthritis, hepatoprotective, wounds, ulcers, rheumatism arthritis, scabies, anthelmintic, and leprosy in traditional Indian medicine. [17]. Pongamia Pinnata L. contains a lot of prenylated flavonoids, like furanoflavones, furanoflavonols, chromenoflavones, furanochalcones, and pyranochalcones, as well as alkaloids, carbohydrates, and reducing sugars. [18,19]

It has been claimed to have anti-diabetic [20], antipyretic [21], anti-microbial [22], anti-inflammatory [23], anti-plasmodial [24] and other properties. Annona Squamosa (Annonaceae) is well-known in English as Custard Apple and in Hindi as Sharifa [25]. This plant is supposed to have a wide range of healing characteristics [26]. Several researchers have looked into its potential as an insecticidal agent [27]. Annona squamosa leaf extracts were found to have free radical scavenging activities [28]. Annona squamosa leaf extract has been shown to have hypoglycemic and anti-diabetic properties [29, 30]. A bioactive acetogenin with anticancer potential [31, 32] has been isolated from the bark of Annona squamosa. This plant yielded flavonoids from the leaves [33], apomorphine alkaloids [34], glycoside [35], and squamoline [36].

Materials and Methods

Animals

Adult who is in good shape Following Institutional Ethics Committee permission, wistar rats of either sex weigh up 200 to 300 gr. remained used. They were kept in a typical laboratory environment with a temperature of 25^{o}C ± 2°C and 12 hour light/dark cycles. The rats stayed given unlimited amounts of rat food & water. Animals were given a seven-day acclimatisation period before being used in tests.

Drugs

Novartis Pharma Company provided Rifampin and Isoniazid. The dosages employed in this investigation were (100mg/kg body weight) &(50mg/kg body weight), correspondingly. INH & RIF were given orally used for 21 days in a row [37].

Extraction of plant material

Pongamia Pinnata

500 g of dried Pongamia Pinnata powder was extracted in a Soxhlet apparatus through ethanol as a solvent. The extract was concentrated to a semisolid stability on a rotary flash evaporator & then dried over a water bath. The extracted extract produced 72 g. The dose was set at 400 mg/kg body weight.

Annona Squamosa

In a soxhlet apparatus, 500 g of Annona Squamosa dried powder was extracted with ethanol as the solvent. The extract was concentrated to a semisolid consistency using a rotary flash evaporator before drying in a water bath. The extraction yielded 72 g of extract. The dose was set to be 400 mg/kg of body weight.

Chemicals

Silymarin, All kits (SGPT, SGOT, Malondialdehyde (MDA), Superoxide Dismutase (SOD)), were achieved from Sigma Company, USA.

Experimental Proposal

The test animals be present divided into five groups, each through six animals, and the subsequent is the treatment regimen for the following 21 days.

Group - 1: Normal Control (0.9% Normal Saline solution 1ml/kg., orally)
Group - 2: Toxic control (Isoniazid (27mg/kg) + Rifampin (40mg/kg), orally)
Group - 3: Standard (Silymarin (100mg/kg) orally) + administration of INH & RIF later 1hr.
Group - 4: Pongamia Pinnata (400mg/Kg, orally) + administration of INH&RIF later 1hr.
Group - 5: Annona Squamosa (400mg/Kg, orally) + administration of INH&RIF later 1hr.
On the 21st day, heart puncture under ether anesthesia was used to obtain blood samples from all of the groups of animals. The livers of the animals were extracted when they were sacrificed for histopathological analysis and biochemical parameter analysis.

Evaluation of Liver Injury

Gross morphological examination

The rats' livers remained removed and washed with normal saline. Subsequently blotting through filter paper, they were weighed. The indicators of the liver be there considered as a proportion of body weight. [38] Afterwards, using Mitchell et al. qualitative method, a gross morphological examination of liver lesions was implemented. [39] They were given the following grades:

0: No abnormalities
1+: Lesser injury
2+: Slight to moderate injury
3+: Serious injury.
For exclusively liver was cut as 2 halves. The Right lobe had soaked in isotonic 10% protected formalin preservative used aimed at histological evaluation, whereas the Left lobe was washed with cold Physiological Saline & formerly standardized through cool phosphate buffer saline on behalf of MDA & SOD testing.

Biochemical Estimation

The Reitman & Frankel method was used to calculate Serum Glutamate Pyruvic Transaminase (SGPT) & Serum Glutamate Oxaloacetic Transaminase (SGOT). [40] Tissue MDA & SOD action were determined using the Biuret technique. [41, 42]

Histological Examination

To make cutting easier, liver samples stood extracted, soaked in formalin, dried out, cleaned, saturated, & embedded in paraffin. Histopathological alterations were assessed using haematoxylin and eosin [43].

Statistical Examination

The consequences were presented as mean ± standard error of the mean. On behalf of the study, a one-way ANOVA was employed, monitored through a suitable post hoc test (Tukey's test). $P \leq 0.05$ was deemed statistically considerable.

Results

The average body and liver weights for each group are shown in Table 1. When treated rats with isoniazid and rifampin suspension were compared to matched control rats, there was a substantial loss in body weight & a rise in liver weight. Pongamia Pinnata, Annona Squamosa, and silymarin treatment resulted in a significant rise in body weight while reducing liver weight.

Gross Morphological Examination

Following a qualitative examination of the liver gross morphology [Table 2], the severity of liver necrosis was determined. Annona Squamosa considerably reduced degeneration & tissue necrosis in the liver when associated to Group -2, according to gross morphological scores. However, as associated to Group - 5, the hepatoprotective activity was not statistically substantial [Table 2].

Histopathological Examination

The liver slices of rats cured with vehicle demonstrated common hepatic architecture in histological examinations [Figure - A]. The management of anti-tubercular medicines to Group 2 for 21 days resulted in alterations in

inflammation, degeneration &necrosis on histological consideration of rat livers [Figure - B]. Its consequence in reversing cell destruction &infiltration was equivalent towards Silymarin [Figure - C].Co-administration of Pongamia Pinnata& Annona Squamosa by means of anti-tubercular medicines corrected histological alterations for example inflammation, degeneration and necrosis [Figure – D & E].

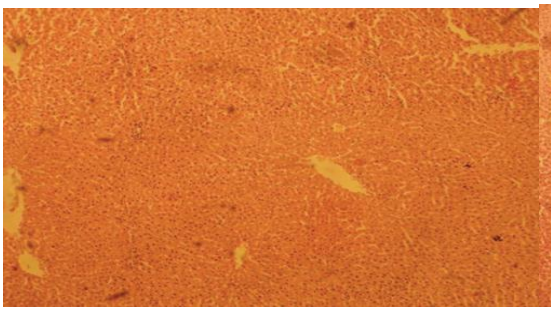

Figure - A: Normal Control – Normal Saline

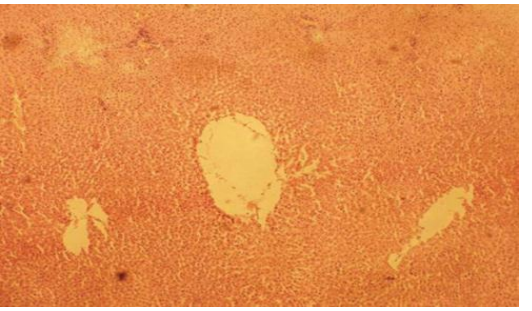

Figure - B: Toxic Control (INH & RIF)

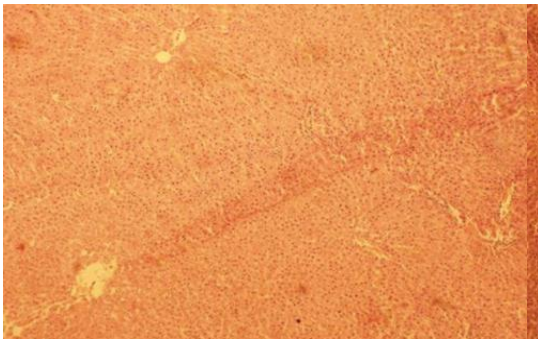

Figure - C: Standard (Silymarin) + INH & RIF

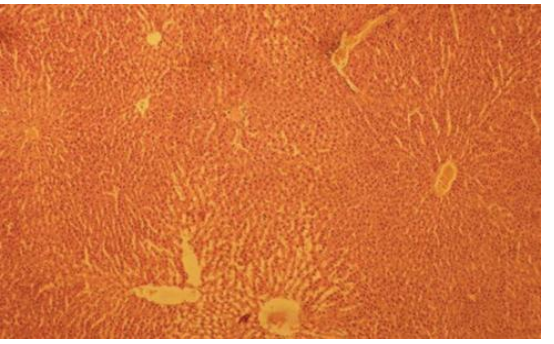

Figure - D: Pongamia Pinnata + INH & RIF

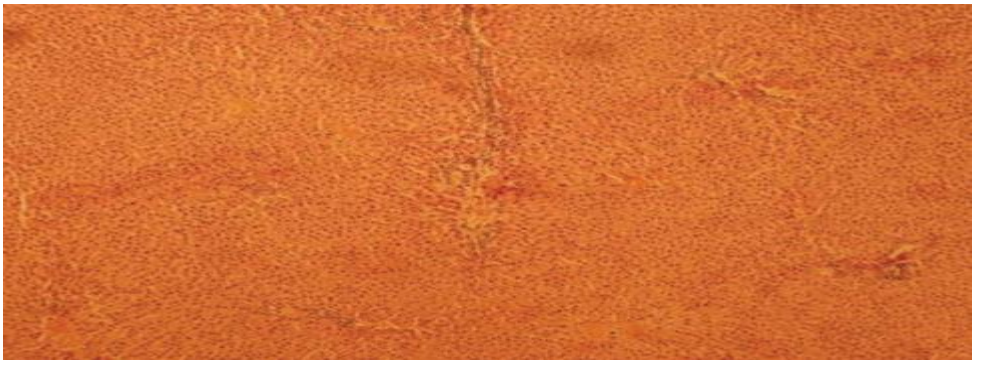

Figure - E: Annona Squamosa +INH & RIF

Estimates of biochemistry

Serum SGPT and SGOT

Anti-tubercular medicines produce significant hepatotoxicity & tissue damage in the rat liver. As a result, there is a rise in the SGPT & SGOT. In this investigation, Group - 2, which expected anti-tubercular medications for 21 days, had a substantial increase in the SGPT & SGOT when associated to the control group [Table - 2]. Management through Pongamia Pinnata and Annona Squamosa together by anti-tubercular medications (Group – 4 & 5) for 21 days considerably restored blood SGPT ($P < 0.01$) & SGOT ($P < 0.01$) levels when related to anti-tubercular medication usage Group - 2.

MDA and SOD activity

After 21 days of treatment, Table - 2 indicates that the activity of several pharmacological involvements on (MDA) points in rats. MDA levels were substantially higher ($P < 0.05$) in Group - 2 (52.61±5.33) than in the healthy control rats (46.32±9.18). When compared to the healthy and INH + RIF suspension groups, administration of Pongamia Pinnata at a dosage of 400mg/kg was somewhat effective in reversing the increase in MDA levels. Similarly, Annona Squamosa & silymarin were discovered to be more successful in cure than Group - 2. However, when compared to Annona Squamosa and silymarin, the activity of Pongamia Pinnata was not statistically important. When associated to the control group, the level of the antioxidant enzyme SOD decreased significantly in Group - 2. Pongamia Pinnata co-administration with anti-tubercular medicines (Group - 3) elevated SOD levels considerably ($P < 0.05$). The impact, however, was not statistically significant when compared to silymarin and Annona Squamosa.

Table - 1: Body And Liver Weights Of Rats In Various Groups

GROUPS	**Primary body weight (gr.)**	**Final body weight**	**Percent Change**	**Liver Weight**	**Liver Index**
GROUP - 1	335±2.32	361±8.76	8.86	12.46±0.53	4.53
GROUP - 2	302±13.6	369±14.6	-12.32*	12.75±0.54	5.74*
GROUP - 3	209±4.32	234±9.77	13.4#,$	7.92±0.43	4.28
GROUP - 4	250±7.45	275±9.46	11.46#,$	9.65±0.46	4.48
GROUP - 5	319±7.33	336±8.76	7.63#	10.95±0.78	3.36*

Liver index was intended as (liver weight/body weight×100 percent). The results were considered as mean±SEM (*,#,$=$P<0.05$) *when related with vehicle control group, #when compared with anti - TB medicine group, $ when paralleled with silymarin group. One-way ANOVA was used to analyse the data, followed by the Tukey HSD test. HSD stands for "honestly significant difference," and SEM stands for "standard error of the mean."

Table - 2: Assessment Of Various Measured Considerations In Rat Investigational Groups

Bio - chemical Considerations	**Group - 1**	**Group - 2**	**Group - 3**	**Group - 4**	**Group - 5**
SGPT (U/L)	33.01±2.60	187.19±23.27**	41.19±5.62##	51.19±6.85##	46.03±4.03###
SGOT (U/L)	30.31±4.20	61.03±41.72**	42.78±4.85##	61.03±4.32##	47.19±4.00###
MDA (µ mol/ml of tissue homogenate)	46.32±9.18	52.61±5.33*	43.06±3.56#	55.60±5.12#	31.11±5.21###
SOD (U/ml)	38.27±1.18	19.83±1.51*	31.59±1.89#	33.78±1.60#	35.73±1.33#,++
MI (0 – 3)	0	3.12±0.36**	2±0.16##,$$	2±0.36##,$$	0.73±0.43##

The results were expressed as meanSEM (*,# = p0.05;**, ##, $$ = p<0.01) when associated to the vehicle control group, # when associated to the anti-TB medicines group, $ when compared to the silymarin group, + when related to Pongamia Pinnata, and @ when related to Annona Squamosa. One-way ANOVA was used to analyse the data, followed by the Tukey HSD test. SGPT: Serum Glutamate Pyruvic Transaminase, SGOT: Serum Glutamate Oxaloacetic Transaminase, MDA: Malondialdehyde, SOD: Superoxide Dismutase, MI: Morphological Index: HSD stands for "Honestly Significant Difference," and SEM stands for "Standard Error of the Mean."

Discussion

Anti-tubercular drugs like isoniazid, rifampin, and pyrazinamide can cause hepatotoxicity, which is a serious side effect. They are hepatotoxic on their own, and when given together, their toxicity is amplified. Hepatotoxicity caused by anti-tubercular medicines is facilitated by oxidative stress & free radical destruction to hepatocytes. [44]. A rise in serum SGOT & SGPT levels indicates hepatic damage. Lipid peroxidation is caused by free radicals, which raises serum MDA levels. As a result, lipid peroxidation is a marker of severe liver damage because it represents tissue injury caused by inflammation. [45, 46] The most reliable indicator of liver damage is a liver biopsy. Degeneration, necrosis, and fibrosis are signs of liver damage, whereas a decrease in these considerations & evidence of regeneration are signs of hepatoprotection. Anti-tubercular medications also caused inflammation, degeneration & necrotic changes in the rat liver.

Simultaneous administration of Pongamia Pinnata with anti-tubercular medicines expressively reduced the rise in serum SGPT, SGOT, and tissue MDA levels in this study. Similarly, when associated to the group receiving only anti-tubercular medicines, Pongamia Pinnata significantly reduced the drop in serum total protein and SOD. Inflammation, degeneration, and necrotic changes were reduced when Pongamia Pinnata was given. These findings demonstrated that Pongamia

Pinnata can performance as a hepatoprotective agent, preventing hepatotoxicity affected by anti-tubercular drugs.

Pongamia Pinnata and Annona Squamosa have been shown to reduce heavy haemorrhage and hepatocellular necrosis in histopathological studies. Treatment with Pongamia Pinnata and Annona Squamosa normalised the anti-tubercular medicine induced histopathological changes; thus, it is suggested that the hepatoprotective activity of Pongamia Pinnata and Annona Squamosa against anti-tubercular medicine induced hepatotoxicity may be due to its ability to reduce oxidative stress.

Conclusion

When compared to the toxic control, the antioxidant parameters, biochemical parameters, and histopathological studies reveal that Pongamia Pinnata and Annona Squamosa have hepatoprotective activity at 400 mg/kg. It has better results in the treatment of drug-induced liver toxicity than Pongamia Pinnata – Annona Squamosa.

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