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IN-VIVO STUDY OF POLYHERBAL FORMULATION AGAINST ANTITUBERCULAR DRUGS INDUCED HEPATOTOXICITY

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Abstract

Formulations containing polyherbal ingredients are frequently utilized to preserve the liver and cure hepatic dysfunction and regeneration. They can also increase hunger and guard against harm to the gastrointestinal system. The current study's goal was to evaluate the in-vivo hepatotoxicity caused by antitubercular medication formulations. This study examines the hepatotoxicity of isoniazid (INH) and rifampicin (RIF) administered to rat kidneys, as well as the mitigating effects of a polyherbal formulation. Rats were given oral doses of polyherbal extract in addition to INH and RIF to render them drunk. The effect on histopathology was examined in the kidneys of rats. Rats that were not treated exhibited deviation, but after receiving treatment, their weight remained normal. However, the rats receiving polyherbal therapy were able to keep their biochemical indices close to normal. According to histopathological findings, there was not enough INH and RIF-induced hepatotoxicity to cause appreciable tissue damage. Rat kidneys that have received treatment or not show abnormal architecture as a result. In summary, rat kidneys treated with polyherbal medicine are shielded against the hepatotoxic effects of RIF and INH.

Keywords: - Isoniazid, Rifampicin, Polyherbal extract

1. INTRODUCTION

Despite advances in medicine and social and economic growth, tuberculosis remains the second leading cause of death worldwide, caused by a single infectious organism. It claimed 1.4 million lives worldwide in 2010, with 330,000 deaths recorded in India alone.[1,2] Nowadays, the two main chemotherapeutic medications used to treat tuberculosis are isoniazid (INH) and rifampicin (RIF).[3,4] Nevertheless, a number of negative consequences, including neurotoxicity, hematological profile alteration4, renal malfunction, and liver dysfunction, have been documented.[5,6] Hepatotoxicity brought on by the reactive metabolite of INH and RIF is cited as the cause of these negative effects.[7,8]

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2. METHODOLOGY

2.1 *IN VIVO* STUDIES

Antitubercular Induced Hepatotoxicity will be used in the pharmaceutical inquiry. A clear solution will be obtained by dissolving isoniazid (50 mg/kg, p.o.) and rifampicin (100 mg/kg, p.o.) in distilled water (1 ml/kg) and raising the pH to 3 with 0.1 N HCl. The animals will be treated for the upcoming in vivo hepatoprotective investigations as described below. They will be separated into 5 groups, with 5 animals per group.

S.No	Group No.	Description	Dose	Animal per group
1.	Group – I	Saline solution	1 ml (p.o)	5
2.	Group – II	Isoniazid (INH) + Rifampicin (RIF)	50 mg/kg, 100 mg/kg (p.o)	5
3.	Group – III	Isoniazid (INH) + Rifampicin (RIF)+ Silymarin	50 mg/kg (p.o)	5
4.	Group – IV	Isoniazid (INH) + Rifampicin (RIF)+ hydroalcoholic extracts of *Cassia fistula*	200mg/kg (p.o)	5
5	Group – V	INH + RIF + hydroalcoholic extracts of *Boerhaavia diffusa*	240 mg/kg	5
6.	Group-VI	INH + RIF + hydroalcoholic extracts of Polyherbal formulation (PF)	500mg/kg	5

Table 1. Dose of antitubercular drug

2.2 Acute toxicity study

In accordance with OECD-423 guidelines, an acute oral toxicity study was carried out. Each of the five groups of animals consisted of creatures. overnight water-only fasting. Following that, PF were given orally in dosages of 100, 200, 500, 1000, 1500, and 2000 mg/kg. For one hour, the animals were under constant observation. Monitoring was done for general behavioral, neurological, and autonomic profiles for four hours, and then again after twenty-four hours. The dose given was classified as harmful if two to three animals showed signs of death. The hazardous dose was confirmed by repeating the dose if one animal showed signs of mortality.

Following a 24-hour period, samples were obtained, and the levels of urea, creatinine, AST, ALT, ALP, and physical activity were determined along with the rate of hair loss. The in vivo trial was split into phases III and IV, each lasting twenty-one days, after the dosage was fixed.

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2.3 Evaluation of hepatoprotective and antioxidant properties of polyherbal formulation and SIL (silymarin) on INH alone induced hepatotoxicity

This paradigm was used to assess the hepatoprotective and antioxidant effects of SIL and polyherbal formulation against sub-acute INH alone-induced hepatotoxicity in rats. Furthermore, research was conducted to examine the effects of INH alone on the vital lipid parameters (TL, TG, CHO, PL, and FFA) in plasma, liver, and adipose tissue, as well as the prevention of these changes through the use of polyherbal formulation and SIL.

S.No	Group No.	Description	Dose
1.	Group-I	Saline solution	1 ml
2.	Group-II	INH alone	50 mg/kg
3.	Group-III	Isoniazid (INH) + Silymarin+ hydro-alcoholic extracts of Polyherbal formulation (PF)	200 mg/kg
4.	Group-IV	hydroalcoholic extracts of Polyherbal formulation (PF)	500mg/kg
5.	Group-V	Silymarin (SIL)	50mg/kg

Table 2. Evaluation of hepatoprotective and antioxidant properties of polyherbal formulation and SIL (silymarin) on INH alone induced hepatotoxicity

2.4 Evaluation of hepatoprotective and antioxidant properties of Polyherbal formulation and SIL on combined treatment of Antitubercular drugs (INH+RIF) induced hepatotoxicity

The present study assessed the hepatoprotective and antioxidant properties of polyherbal formulation and SIL in rats treated with a combination of antitubercular medicines (INH+RIF) to mitigate hepatotoxicity. Furthermore, research was conducted to examine the changes in essential lipid parameters (TL, FG, CHO, PL, and FFA) in plasma, liver, and adipose tissue caused by the combined use of antitubercular drugs (INH+RIF) and how SIL and polyherbal formulations prevented these changes.

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S. No	Group No.	Description	Dose
1.	Group-I	Saline solution	1 ml
2.	Group-II	INH+RIF alone	50 mg/kg
3.	Group-III	Isoniazid (INH) + RIF+ Silymarin	250 mg/kg
4.	Group-IV	Isoniazid (INH) + RIF + hydro-alcoholic extracts of Polyherbal formulation (PF)	200mg/kg
5.	Group-V	hydroalcoholic extracts of Polyherbal formulation	500mg/kg
6.	Group-VI	Silymarin (SIL)	50mg/kg

Table 3. Evaluation of hepatoprotective and antioxidant properties of polyherbal formulation and SIL on combined treatment of antitubercular drugs (INH+RIF) induced hepatotoxicity

2.5 BLOOD AND TISSUE SAMPLES PROCESSING

2.5.1. Serum preparation

Blood was drawn into a test tube that was dry. Blood is separated using a centrifuge that runs at 3000 rpm for ten minutes.

2.5.2. Plasma preparation

The blood, drawn into a centrifuge tube that has been heparinized. After centrifuging it for ten minutes at 3000 rpm, the plasma was separated.

2.5.3. Erythrocyte preparation

Physiological saline was used to wash the erythrocytes after the plasma was separated. The erythrocyte was lysed at pH 7.4. After this hemolysate was separated, the amount of enzymic antioxidants was estimated using the supernatant.

2.5.4. Lipid extract preparation

By homogenizing the tissues to a predetermined weight of 250 mg, the lipids were removed.

The measurement of TC, FFA, TG, and PL was then performed using an aliquot of this extract.

2.5.5 Tissue sampling for histopathological study

Three rats were employed in the research of histopathology. The liver was promptly removed and preserved in 10% formalin.

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2.5.6 Hepatocyte preparation

After chopping the liver into tiny pieces, Hank's balanced salt solution was used to wash it.

following sequential filtration through 100 and 40 mm mesh.

2.6 ESTIMATION OF LIVER MARKERS

2.6.1 Assay of AST

0.1 mL of serum was mixed with 0.5 mL of buffered substrate. Instead of adding serum to the blank tubes, 0.1 mL of distilled water was added. Two drops of aniline citrate reagent and 0.5 mL of DNPH reagent were added an hour later, and the mixture was left to sit at room temperature for 20 minutes. 5.0 mL of 0.4 N NaOH was added last. After 10 minutes, a set of standards that were handled similarly were read at 520 nm.

2.6.2 Assay of ALT

The process followed the same guidelines as the aspartate transaminases assay.

3. RESULTS

3.1 ACUTE TOXICITY STUDIES

Rats in the current study did not experience any negative side effects or mortality at concentrations of polyherbal formulation extract (100, 200, 500, 1000, 1500, and 2000 mg/kg b.w.).

S/No.	No. of Animals	Extract Dose (mg/kg)	Casualty/ Mortal
1.	5	100	0
2.	5	200	0
3.	5	500	0
4.	5	1000	0
5.	5	1500	0
6.	5	2000	0

Table 4. Acute Toxicity of formulation

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3.2 Evaluation of hepatoprotective and antioxidant properties of polyherbal formulation against antitubercular induced hepatotoxicity

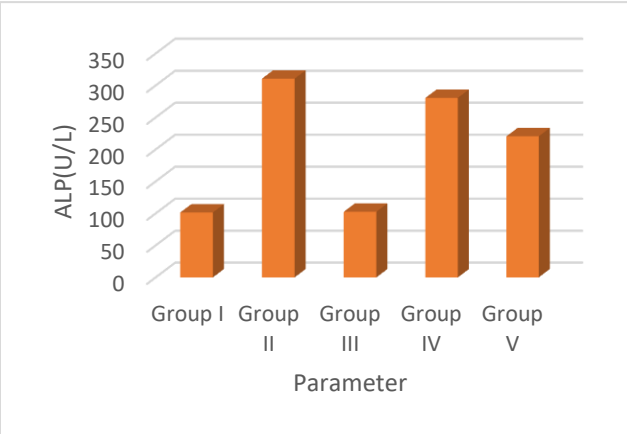

Figure 1. ALP

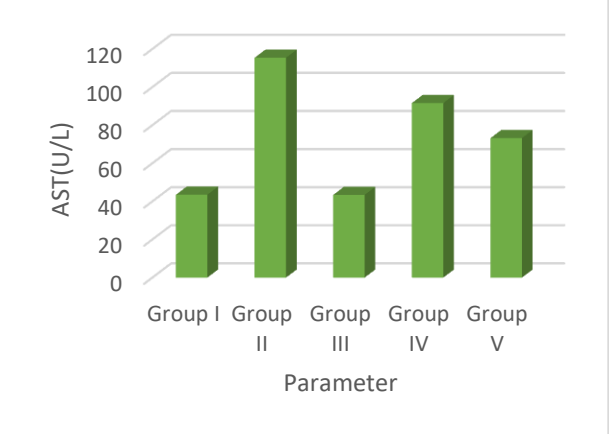

Figure 2. AST

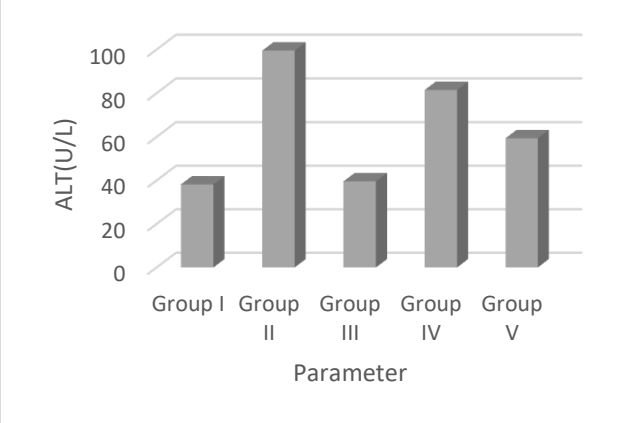

Figure 3. ALT

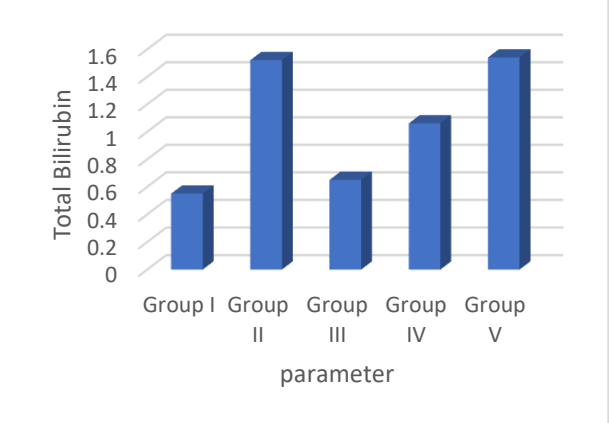

Figure 4. Total Bilirubin

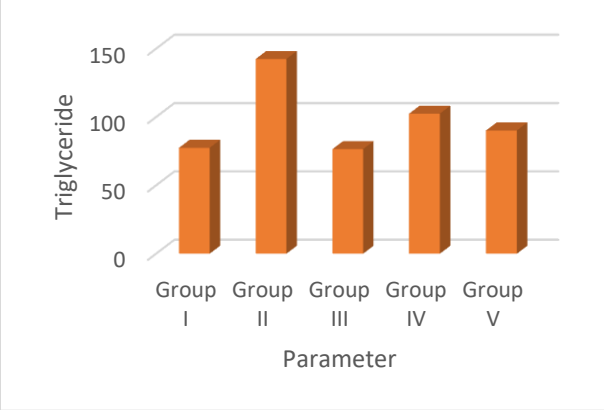

Figure 5. Triglyceride

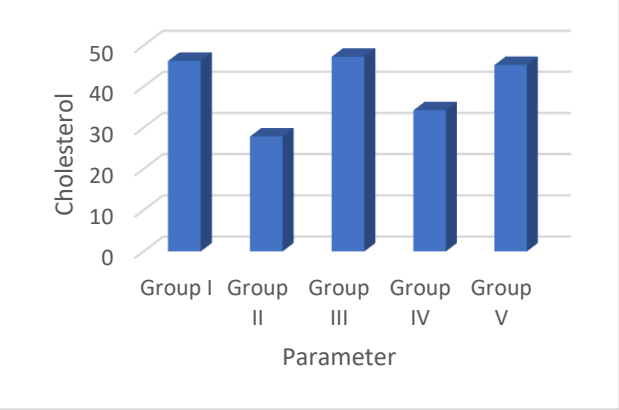

Figure 6. Cholesterol

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3.3 Effect of Leaf Extract on antitubercular Induced hepatotoxicity in Lipid metabolism

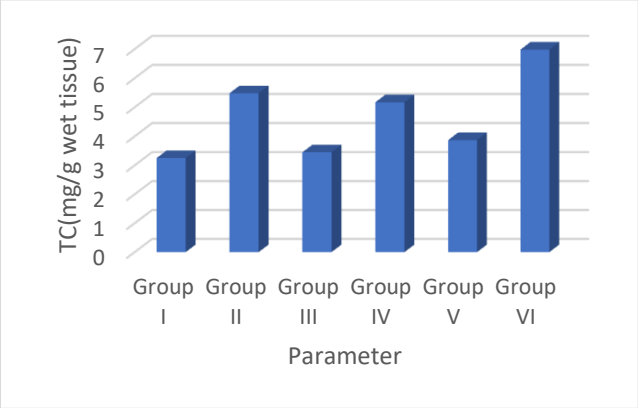

Figure 7. Effect of polyherbal extract on TC

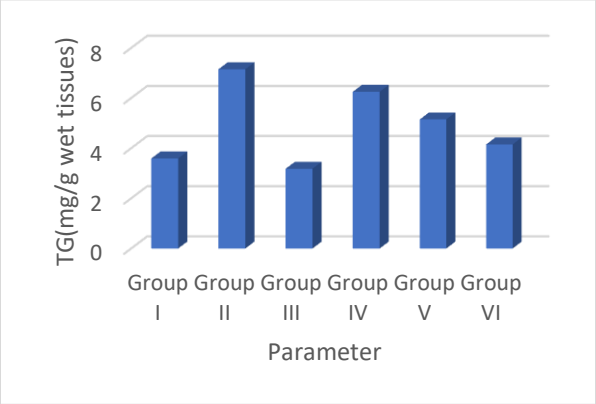

Figure 8. Effect of polyherbal extract on TG

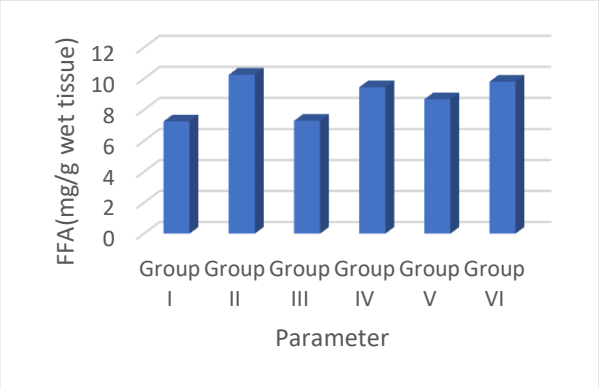

Figure 9. Effect of polyherbal extract on FFA

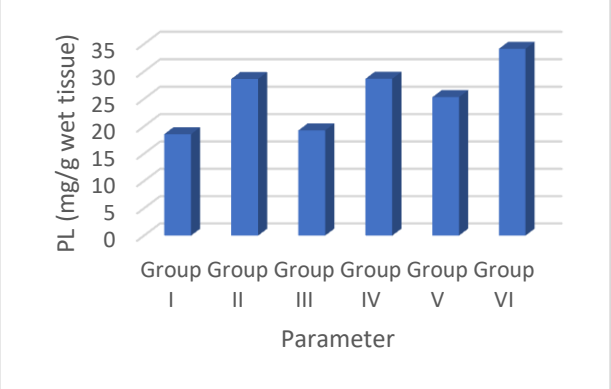

Figure 10. Effect of polyherbal extract on PL

3.4 Evaluation of hepatoprotective and antioxidant properties of polyherbal extract and SIL on its simultaneous treatment against INH alone induced hepatotoxicity

The treatment with hydroalcoholic extract of polyherbal extract (200 and 500 mg/kg) showed the reduced massive fatty changes compared to intoxicated rats. The treatment with hydroalcoholic extract of polyherbal extract (500 mg/kg b.w) and its isolated fraction reduced the morphological changes.

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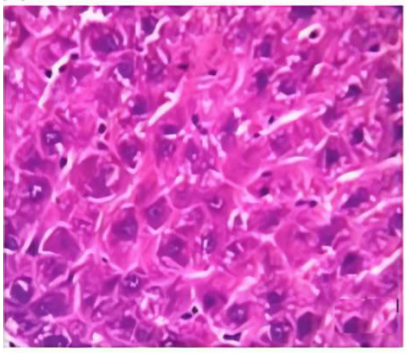

Figure 11. Group I

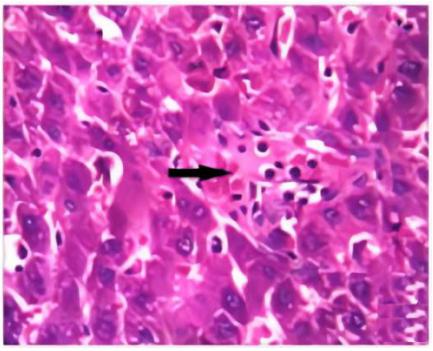

Figure 12. Group II

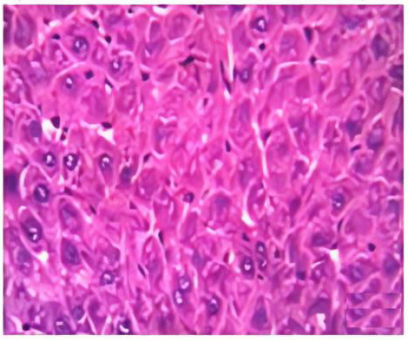

Figure 13. Group III

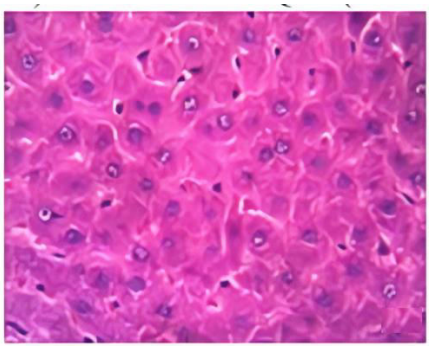

Figure 14. Group IV

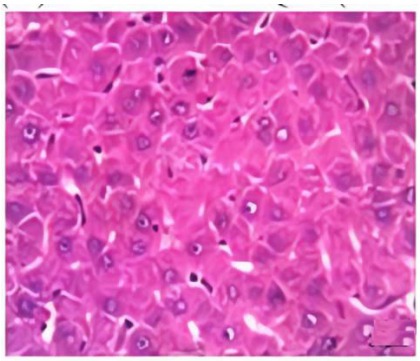

Figure 15. Group V

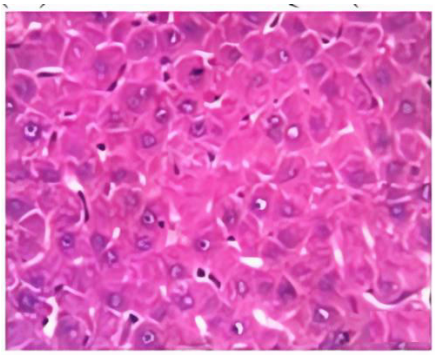

Figure 16. Group VI

4. SUMMARY

More functions are assigned to the liver than to any other human organ, making it the primary glandular organ in the body. An excellent source of information for this field of study would be medicinal plants utilized in traditional folk medicine. Finding the medicinal foundation of a polyherbal extract on antitubercular-induced toxicity in rats is the aim of the investigation. Higher quantities of the extract showed abnormal physical alterations but no behavioral impairments, according to the acute toxicity research. Antitubercular was employed in this investigation to produce hepatotoxicity. The various polyherbal extract concentrations were

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administered to antitubercular-induced groups. As a typical medication, silymarin was utilized to control hepatotoxicity in a specific group of rats. The polyherbal extract's hepatoprotective and antioxidant properties were contrasted with those of the silymarin-treated groups. Since antitubercular is a well-known hepatotoxin, antitubercular-induced liver damage control was shown to be associated with a notable increase in the activity of hepatic marker enzymes such as AST, ALT, GGT, and ALP. According to the findings, rats given polyherbal extract had elevated levels of these markers that were almost at normal levels. Accordingly, the findings showed that the polyherbal extract was advantageous to antitubercular-induced rats and that it might be applied as a phytomedicine to treat liver disease.

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