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Research Article

PROTECTIVE EFFICACY OF *TRIDAX PROCUMBENS* AND *FICUS RELIGIOSA* AGAINST ISONIAZID AND RIFAMPICIN INDUCED HEPATOCELLULAR DAMAGE IN RATS: A PRELIMINARY COMPARATIVE EVALUATION ON BIOCHEMICAL PARAMETERS

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ABSTRACT

Objective: There are limited therapies available for prevention and treatment of hepatitis following antitubercular therapy. Very few reports are available regarding the hepatoprotective activity of *Tridax procumbens* (*Tridax* daisy) and *Ficus religiosa* (Aswatha) against the hepatitis induced by antitubercular drugs. Hence, we studied the hepatoprotective activity of *Tridax* and *Ficus* in isoniazid- and rifampicin-induced hepatitis in rats.

Methods: The rats were divided into five groups. Group 1 - Control, Group 2 - Toxic control received isoniazid + rifampicin (100 mg/kg intraperitoneal each), Group 3 - Received *Tridax* extract (200 mg/kg p.o.) along with isoniazid and rifampicin, Group 4 - Received *Ficus* extract (200 mg/kg p.o.) with isoniazid and rifampicin, Group 5 - Standard group receiving Liv-52 (10 mg/kg p.o.) along with isoniazid and rifampicin. After 21 days, alkaline phosphatase (ALP), alanine transaminase, aspartate transaminase, and bilirubin levels were estimated from the serum. One-way ANOVA was applied to test for significance of biochemical data of the different groups. The significance is set at $p \leq 0.05$. As evident from our study, rats receiving *Tridax* shows significant improvement in all liver function test (LFT) parameters.

Results: There is a significant difference in all the LFT parameters between *Tridax* group and Liv-52 group (p=0.004 for total bilirubin, 0.001 for ALP). Between Liv-52 group and *Ficus* group only significant in total bilirubin (p=0.04).

Conclusion: From this study, it can be concluded that methanolic extract of both *Tridax* and *Ficus* has got significant hepatoprotective effect.

Keywords: Isoniazid, Rifampicin, Liver function tests, Hepatoprotective.

INTRODUCTION

The liver's most important task is to filter toxic substances from the body including alcohol and many different medications [1]. In the 21st century new insight is given to herbal management of many clinical conditions including liver diseases [2]. The first line antitubercular drugs, namely, rifampicin, isoniazid and pyrazinamide are potentially hepatotoxic drugs. These drugs are metabolized by the liver. Adverse effects of antitubercular therapy are sometimes potentiated by multiple drug regimens. Thus, though isoniazid, rifampicin and pyrazinamide each in itself are potentially hepatotoxic, when given in combination, their toxic effect is further enhanced [3].

Tridax procumbens and *Ficus religiosa (Aswatha)* are the herbs found throughout India are employed as indigenous medicine for a variety of ailments including jaundice [4]. However, very few reports are available regarding the hepatoprotective activity of *T. procumbens* and *F. religiosa* (*Aswatha*) against the hepatitis induced by antitubercular drugs. Keeping this fact in view, this study was undertaken to investigate the hepatoprotective activity of *Tridax and Ficus*, against isoniazid- and rifampicin-induced hepatic damage in albino rats.

METHODS

This study was conducted in the Department of Pharmacology. It was approved by Institutional Animal Ethics Committee of KIMS, KIIT University (KIIT/KIMS/IAEC/03).

Plant material and preparation of extract

The leaves of F. religiosa and *T. procumbens* were freshly collected in the month of May-June 2015 in and around of Bhubaneswar, Odisha. The leaves were washed with water, shade-dried, ground to a moderately coarse powder. The powdered leaves were subjected to extraction by refluxing with methanol in a soxhlet extractor for 72 hrs. The resultant extract was evaporated to dryness.

Liver function tests (LFT)

Bilirubin, alkaline phosphatase (ALP), alanine transaminases (ALT), and aspartate transaminases (AST) were assayed using standard reagent kits (Accurex) with Photometer 5010.

Animals

Male Wistar albino rats, weighing about 150-200 g obtained from Animal House in the Department of Pharmacology, were used for the experimental study. The rats were maintained in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, India. Rat cages had the provision of food and water. Those rats having LFT parameters in normal range were included in the study. Normal LFT range of rats is:

- AST (serum glutamic oxaloacetic transaminase [SGOT]) - 45.7-80.8 U/L
- ALT (serum glutamic-pyruvic transaminase [SGPT]) - 17.5-30.2 U/L
- ALP - 56.8-128 U/L
- Total bilirubin - 0.2-0.55 mg/dl.

Animals were kept in the animal house at an ambient temperature of 25°C and 45-55% relative humidity, with 12 hrs each of dark and light cycles.

Hepatotoxicity induced by isoniazid and rifampicin

Isoniazid and rifampicin solution were prepared separately in sterile distilled water. Rats were injected isoniazid (100 mg/kg) and rifampicin (100 mg/kg), both intraperitoneal (i.p.) for 21 days.

Liv-52 (10 mg/kg) was used by orally as a standard drug in this study.

Rats were divided into five different groups (n=6):

- Group 1 - Control
- Group 2 - Toxic control receive isoniazid + rifampicin (100 mg/kg i.p. each)
- Group 3 - Received *Tridax* extract (200 mg/kg p.o) along with isoniazid and rifampicin
- Group 4 - Received *Ficus* extract (200 mg/kg p.o) with isoniazid and rifampicin
- Group 5 - Standard group receiving Liv-52 (10 mg/kg p.o) along with isoniazid and rifampicin.

Rats were treated as per the treatment protocol. Body weights of these rats were monitored sequentially in the control and experimental animals for the period of 21-day.

Estimation of parameters

After 21 days, the blood was collected by retro-orbital venous plexus of the rats from each group and sent for estimation of different biomarkers. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. ALP, ALT, AST and bilirubin levels were estimated from the serum using standard kits.

Statistics

All values were expressed as means ± standard deviation (n=6 in each group). One-way ANOVA was applied to test for significance of biochemical data of the different groups. Significance is set at p≤0.05.

RESULTS

In this study we used one-way ANOVA for statistical significance and TUKEY'S comparison test for post hoc analysis.

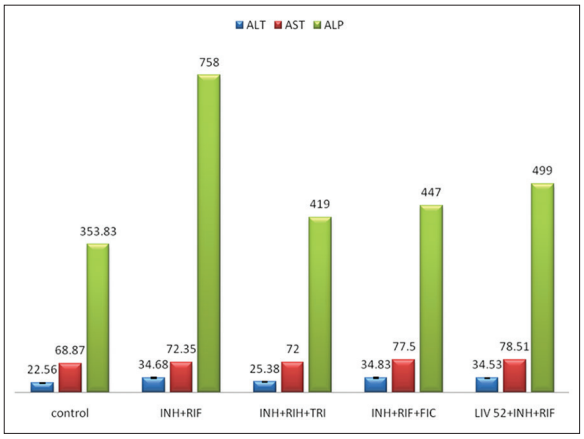

Fig. 1: Mean liver function test parameters in all groups (INH - Isoniazid, RIF - Rifampicin, TRI - Tridax, FIC - Ficus, ALT - Alanine transaminase, AST - Aspartate transaminase, ALP - Alkaline phosphatase)

DISCUSSION

This animal study was conducted in the Department of Pharmacology, KIMS after obtaining the approval from Animal Ethics Committee. We carried out this study to assess the hepatoprotective effect of methanolic extracts of *Tridax* and *Ficus*.

T. procumbens herb is a common herb in India. The whole plant or parts of the plant reported to be used for various ailments such as dysentery, diarrhea, and hair loss [5,6]. Studies have shown also properties such as anti-inflammatory, hepatoprotective, wound healing, antimicrobial, antiseptic, and hypotensive effects [7-11]. A some of the earlier studies have shown the principles present in the plant are dexamethasone, luteolin, glucotureoline, beta-sitosterol, flavones, glycoside, quercetin [12-15]. There are very few studies available regarding hepatoprotective efficacy. Soni *et al.* in the review on herbal hepatoprotective plants focused on the importance of *T. procumbens* in carbon tetrachloride induced liver injury [16]. One study by Parameswari *et al.* has shown the hepatoprotective effect of *Ficus* (methanolic extract) against INH-RIF and paracetamol-induced hepatotoxicity [17]. Another study by Hemalatha suggested the hepatoprotective effect and antioxidant potential of *Tridax* [18]. A study by Wagh and Shinde showed the hepatoprotective effect of varying doses of *Tridax* in paracetamol-induced liver injury [19]. There is evidence of use of *T. procumbens* in African traditional health care for those patients having liver problems [20]. The composition of Liv-52 is well known and it is available in tablets and syrup forms [21]. Numerous studies have been done on Liv-52 regarding its efficacy in hepatitis, alcohol liver disease, cirrhosis, fatty liver, etc. [22]. This is made according to Ayurvedic principles and generally lack toxicity and has efficacy. Its ingredients act synergistically and so has many advantages.

As evident from Table 1, Group 3 receiving *Tridax* shows significant improvement in all LFT parameters compared to Liv-52 group, i.e., ALP (p=0.001), SGOT (p=0.03), SGPT (p=0.003), direct bilirubin (p=0.022). There is significant difference between *Tridax* group and *Ficus* group in total bilirubin (p=0.004), direct bilirubin (p=0.02), SGOT (p=0.04), and SGPT (p=0.003). *Ficus* group also shows improvement of LFT parameters, i.e., total bilirubin (p=0.044) compared to Liv-52 but not significant in other parameters as compared to Liv-52 group. The mean liver function test parameters of all the groups are shown in Figure 1. From our study, it is evident that efficacy of *Tridax* is better as a hepatoprotective drug. This may be due to the probable mechanism suggested by Hemalatha according to which it has been demonstrated that *T. procumbens* possibly activates muscarinic cholinergic receptors which also protects the liver via efferent vagus nerve. Zambare *et al.* did pharmacognostic studies and a preliminary phytochemical analysis on *T. procumbens* [23]. However, further studies need to be carried out to study the possible pathway. Further, hepatoprotective effect of *Ficus* as suggested by Parameswari *et al.* may be due to its chemical constituent like flavonoids and phenolic compound. Traditionally, flavonoids produce antioxidant activity so this mechanism suggesting that the

Table 1: LFT parameters in each group

Mean value	Group 1	Group 2	Group 3	Group 4	Group 5	p value
ALP	353.833±91.80	758±91.93 a=0.00	419±71.50 b=0.001	447±97.45 b=0.003	499±60.54 a=0.003, b=0.002, c=0.001	0.000
Total bilirubin	0.40±0.089	1.1±0.16 a=0.000, c=0.000, d=0.009	0.55±0.14 b=0.000, d=0.004	0.65±0.10 c=0.004	0.87±0.10 d=0.044	0.000
Direct bilirubin	0.12±0.041	0.60±0.08 a=0.012, c=0.011, d=0.049	0.22±0.07 b=0.011, d=0.022	0.42±0.12 a=0.04, b=0.049, c=0.022	0.40±0.08 a=0.04, b=0.04	0.000
AST	68.87±7.263	72.35±11.13	72±4.43 d=0.011	77.50±4.61	78.51±3.51 b=0.012, c=0.03	0.020
ALT	22.56±3.08	34.68±2.138 a=0.000, c=0.003	25.38±2.59 d=0.002	34.83±5.43 a=0.001, c=0.048	34.53±4.90 a=0.000, c=0.003	0.000

a: Significant difference with Group 1, b: Significant difference with Group 2, c: Significant difference with Group 3, d: Significant difference with Group 4, LFT: Liver function test, ALP: Alkaline phosphatase, AST: Aspartate transaminase, ALT: Alanine transaminase

extract of the plant may be useful to prevent the oxidative stress induced damage in the liver. Vedulla *et al.* isolated the active principle of *F. religiosa* Linn. by phytochemical test and the compound identified was stigmasterol [24].

In Revised National Tuberculosis Control Programme, patient due to severe hepatitis following antitubercular therapy has to stop therapy or treated with a long-term alternate regimen. We suggest that *Tridax* can be given along with antitubercular therapy that may avoid hepatotoxicity but studies on acute and chronic toxicity profile of *Tridax* is required. One limitation of our study is that we did not focus on acute and subacute toxicity of *Tridax* and *Ficus*.

CONCLUSION

From this study, it can be concluded that both *T. procumbens* and *F. religiosa* methanolic extract have got hepatoprotective effect. There is a significant difference between hepatoprotective effect of *Tridax* as compared to *Ficus* and Liv-52. More studies required to further prove the toxic profile of *Tridax* and *Ficus* so that there can be better alternative in prophylactic and therapeutic purpose for isoniazid and rifampicin-induced hepatitis.

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