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Original research article

Hepatoprotective Activity of Sida Veronicaefolia against RIF+INH induce Hepatotoxicity in Experimental Animals

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Abstract:
In the present investigation, rats will be given the liver-damaging drug RIF+INH, and the methanol (MESV) extract of the leaves of Sida veronicaefolia will be tested to see how well it protects the rats' livers from damage caused by RIF+INH. The hepatoprotective effects of MESV at a dose of 250 & 500 mg/kg were estimated by using liver function tests and serum profiles. According to the results, the extracts of Sida veronicaefolia at a dose of 250 & 500 mg/kg, not only have a strong hepatoprotective effect by lowering the levels of serum transaminases (SGPT and SGOT), alkaline phosphate, and total bilirubin in the blood, but they also increase the levels of total protein by a lot. The effects of MESV were very similar to those of silymarin, which is given as standard drug treatment.

Introduction

There has been a surge of research over the last three decades suggesting that herbal medicine may prevent, halt, or even reverse the onset of chronic diseases. **(Mehrola,1990)**. Herbal medicine continues to be the first line of therapy for between 75%-80% of the global population, the vast majority of who reside in underdeveloped countries, since it is more culturally acceptable, more compatible with the human body, and has less harmful effects. There has been a clear increase in the usage of their services in recent years in developed countries like Germany, France, the European Union, and the United States.

India belongs to more than 45,000 plant species, and several numbers of them have been studied for their potential therapeutic uses. The Indian government has created a legislative framework for regulating the production, distribution, and use of herbal medicine. Today, there are over 4,000, 000 ayurvedic doctors on record with the government. About one billion dollars, plus another eighty million dollars in exports, comes from the selling of herbal medicine in India as over-the-counter goods, ethical and traditional formulation, and containing medicines from the ayurveda, unani, and siddha systems. Despite India's wealth of herbal medicine resources, the country earns very little from exports to industrialized nations since such exports often consist only of raw materials rather than finished products. **(Sapna Shrikumar, 2007)**

The liver's natural healing process is aided by several herbal medications used to treat a wide range of liver problems. Many different medicinal herbs and products derived from those plants are used in traditional and folk medical practices throughout India to treat liver disorders. The World Health Organization estimates that liver sickness takes the lives of roughly 18,000 persons yearly. The absence of different liver protective drugs is one of the most important factors leading to the incidence of serious liver disorders. Common liver illnesses include cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, hepatic failure, and some forms of malignancy, such as hepatoma.

There is a vast range of therapeutic approaches that may be used to cure liver disorders. Allopathic practitioners often use herbal medicine into the treatment of a wide range of liver disorders. There is still a need to find drugs that can prevent liver damage since no effective therapy for liver disorders has been developed so far.

Many plants used in India's traditional and tribal medical practices are either unknown or remain unclear. The medicinal value of these plants is currently unappreciated by the scientific community. The effects of Sida veronicaefolia as a hepatoprotective agent against RIF+INH induced liver injury in rats are the subject of this investigation.

People have been searching for natural remedies to treat a wide range of diseases since ancient times. The hunt for these people is ongoing. In the search for new and effective therapeutic agents, the rational methods and practice of folk medicine provided a useful perspective.

Finding new, effective, and safe hepatoprotective drugs that can be made from local plants is the primary goal of this research. Traditional medicine has often made use of these herbs. However, not nearly enough study has been done on these compounds to characterize their chemical composition, mechanisms of action, or toxicity.

Our major goal is to develop a safe and effective hepatoprotective medication for therapeutic use.

The Sida veronicaefolia plant, of the Malvaceae family, is a common sight in gloomy areas, where it grows languidly. The places where the forest has been cut down or where the grass has been allowed to grow wild in parks and gardens are the most likely to have this weed **(Lutterodt *et al.*, 1988a)**. Rajbala, Bhumibala, Farid buti, and Shaktibala are just few of the various names for it. It may balance the body's yin and yang energies and get rid of any excess vata, pitta, or kapha **(Dash *et al.*, 1991)**.

The Sida veronicaefolia plant is well-liked by rural women all across its native range due to its extensive usage in traditional medicine. In part, this is due to the plant's reputation for reducing both labor time and labor discomfort. Its intended effects include reducing postpartum bleeding and eliminating most of the discomfort associated with giving delivery. Soup produced from this plant is traditionally eaten in the third trimester **(Lutterodt *et al.*, 1988b)**. Lutterodt discovered that pregnant rats exposed to an alcoholic extract of Sida veronicaefolia had spontaneous abortions. Abortifacient effects are shown when a dose is administered orally and given between the 15th and 17th day of pregnancy **(Lutterodt *et al.*, 1988b)**. An active component similar to muscarine has also been hypothesized to exist in the water-soluble portion of an alcoholic extract of Sida veronicaefolia **(Yua *et al.*, 2008)**. Equally well-documented is the plant's leaves' anticancer potential **(Saluja *et al.*, 2012)**. An extensive enthanobotanical analysis of the plant revealed a number of pharmacologically active phytoconstituents, including alkaloids, flavonoids, triterpenoids, phenolic compounds, saponins, amino acids, and protein **(Saluja *et al.*, 2012)**. Since no such action has been reported for any of the aforementioned plant extracts, the present study

set out to evaluate the hepatoprotective potential of a variety of plant extracts.

Method and Materials

Procurement and Authentication of the Plant

The leaves of *Sida veronicaefolia* were gathered from the surrounding region of Alwar, Rajasthan, and then sent to Sunrise University, Alwar, Rajasthan, where they were certified by Dept. of Botany, Sunrise University, Alwar, Rajasthan.

Preparation of extracts of *Sida veronicaefolia*

In a Soxhlet apparatus, 500g of powdered leaves were extracted with solvent in order to increasing polarity. The materials were concentrated by evaporation **(Farnsworth *et al.*, 1966)**.

Animals

Wistar albino rats (150-200 g) was procured from Central Drug Research Institute, Lucknow, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee after scrutinization. Animals were received the drug by oral gavages tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines (CPCSEA, 2003) with regular inspections of rats. The laboratory conditions duly undertaken by registered veterinary practitioner.

Chemicals

All the chemicals and solvents were of analytical grade. Silymarin was obtained as gift sample from Micro Lbs, Goa, India. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India.

Evaluation of hepatoprotective activity by RIF+INH induced hepatotoxicity in rats

The rats, of both sexes, were split up into 4 groups of six each. (n = 6) **(Balakrishnan *et al.*, 2011; Haque *et al.*, 2011)**

- **Group I (Control)**: administered water (5 ml/kg *p.o.*) *o.d.* for twenty one days.
- **Group II (-ve control):** administered water (5 ml/kg, *p.o.*) and RIF+INH (100 ml/kg, i.p.) *o.d.* for twenty one days.
- **Group III (+ve control):** administered the normal medicine silymarin (25 ml/kg, *p.o.*), water (5 ml/kg, *p.o.*) and RIF+INH (ml/kg, i.p.) *o.d.* for twenty one days.
- **Group IV (Test Sample)** administered Hydroethanolic (MESV) extract (250 mg/kg), water (5 ml/kg, *p.o.*) and RIF+INH (100 ml/kg, i.p.) *o.d.* for twenty one days.
- **Group V (Test Sample)** administered Hydroethanolic (MESV) extract (500 mg/kg), water (5 ml/kg, *p.o.*) and RIF+INH (100 ml/kg, i.p.) *o.d.* for twenty one days.

Assessment of hepatoprotective activity

On last day, blood was obtained from animals by puncturing retro orbital plexus. Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including **SGOT & SGPT (Reitman *et al.*, 1957), ALP (Kind *et al.*, 1954), serum bilirubin (Amour *et al.*, 1965) and serum protein (Lowry *et al.*, 1951)** After collection of blood samples, the animals were sacrificed under deep ether anesthesia.

Morphological parameters like weight of animals, weight of liver have also been used to evaluate the protective effect of the drug. Hepatoprotective chemical causes loss in liver weight/100 gm body weight of rats **(Avadhoot *et al.*, 1991; Bhanwra *et al.*, 2000)**.

Histopathology studies

A portion of liver tissue of all the animal groups was excised and then washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48 hrs and then with bovine solution for 6 hrs and were then processed for paraffin embedding. By using a microtome, sections of 5 mm thickness

were taken and stained with hematoxylin and eosin. These sections were examined under light microscope using a magnification of 100X **(Mankani *et al.*, 2005)**.

Statistical Significance

The results of the study were expressed as mean ± SEM, n=6. ANOVA **(Gennaro *et al.*, 1995)** was used to analyze and compare the data, followed by Dunnet's **(Dunnet *et al.*, 1964)** test for multiple comparisons.

Results

Acute toxicity study

There was no mortality found amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 5000 mg/kg. This finding suggests that the *MESV* was safe or non-toxic to rats and hence doses of 250 & 500 mg/kg, *p.o*. were selected for the study.

Effect of *MESV* on serum marker enzyme levels

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and ALP content of hepatotoxic treated groups. In contrast, pretreatment with *MESV* (250 & 500 mg/kg, *p.o*) and silymarin (25 mg/kg, *p.o*) exhibited an ability to counteract the hepatotoxicity by decreasing serum marker enzymes. The results were showed in table no 1.

Effect of *MESV* on biochemical parameters

In hepatotoxic treated groups, there was a significant increase in total bilurubin and significant reduction in total protein content. Whereas, pretreatment with *MESV* (250 & 500 mg/kg, *p.o*) caused significant reduction in total bilirubin and significant increase in total protein. The results were showed in table no. 1

Table no. 1: Effect of *MESV* extracts on serum enzyme and biochemical parameter in RIF+INH induced hepatotoxicity in rats.

Normal	63.0 ± 3.61	165.04 ± 2.80	187.0 ± 8.01	0.48 ± 0.06	9.67 ± 0.24
Induced (RIF+INH)	176.41 ± 8.24*	363.72 ± 8.34*	340.44 ± 7.56*	6.66 ± 8.04*	5.3 ± 0.16*
Standard (Silymarin)	63.52 ± 3.21***	172.80 ± 4.47***	201.29 ± 8.33***	0.52 ± 2.68***	9.62 ± 4.80***
MESV (2500mg/kg)	94.51 ± 5.71***	298.0 ± 4.76***	258.17 ± 5.82***	0.99 ± 2.93***	8.78 ± 5.51**
MESV (500mg/kg)	81.02 ± 3.61***	260.0 ± 4.66***	233.15 ± 6.30***	0.59 ± 4.23***	7.88 ± 4.02**

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with respective control.

Effect of *MESV* on liver weight

RIF+INH intoxicated group of animals, weight of the liver was significantly increased, but it was normalized in *HEESV* (250 & 500 mg/kg, *p.o*) treated groups of animals. A significant reduction in liver sup*p*orts this finding. The results were showed in table no.2.

Table No. 2: Effect of *MESV* extract on liver weight in RIF+INH induced hepatotoxicity in rats.

Treatment/ Dose	**Liver weight (wt/100gm bw)**
Normal	6.84 ± 0.06
Induced (RIF+INH)	8.48 ± 0.28*
silymarin 25mg/kg	7.02 ± 0.48***
MESV (2500mg/kg)	7.74± 0.28***
MESV (500mg/kg)	7.38 ± 0.24***

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with respective control.

Histopathology

Histopathological studies of liver also provided a supportive evidence for biochemical analysis. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in RIF+INH treated (toxic) control group. Both the plant extracts has prevented these histological changes. The results were showed in fig. no. 1.

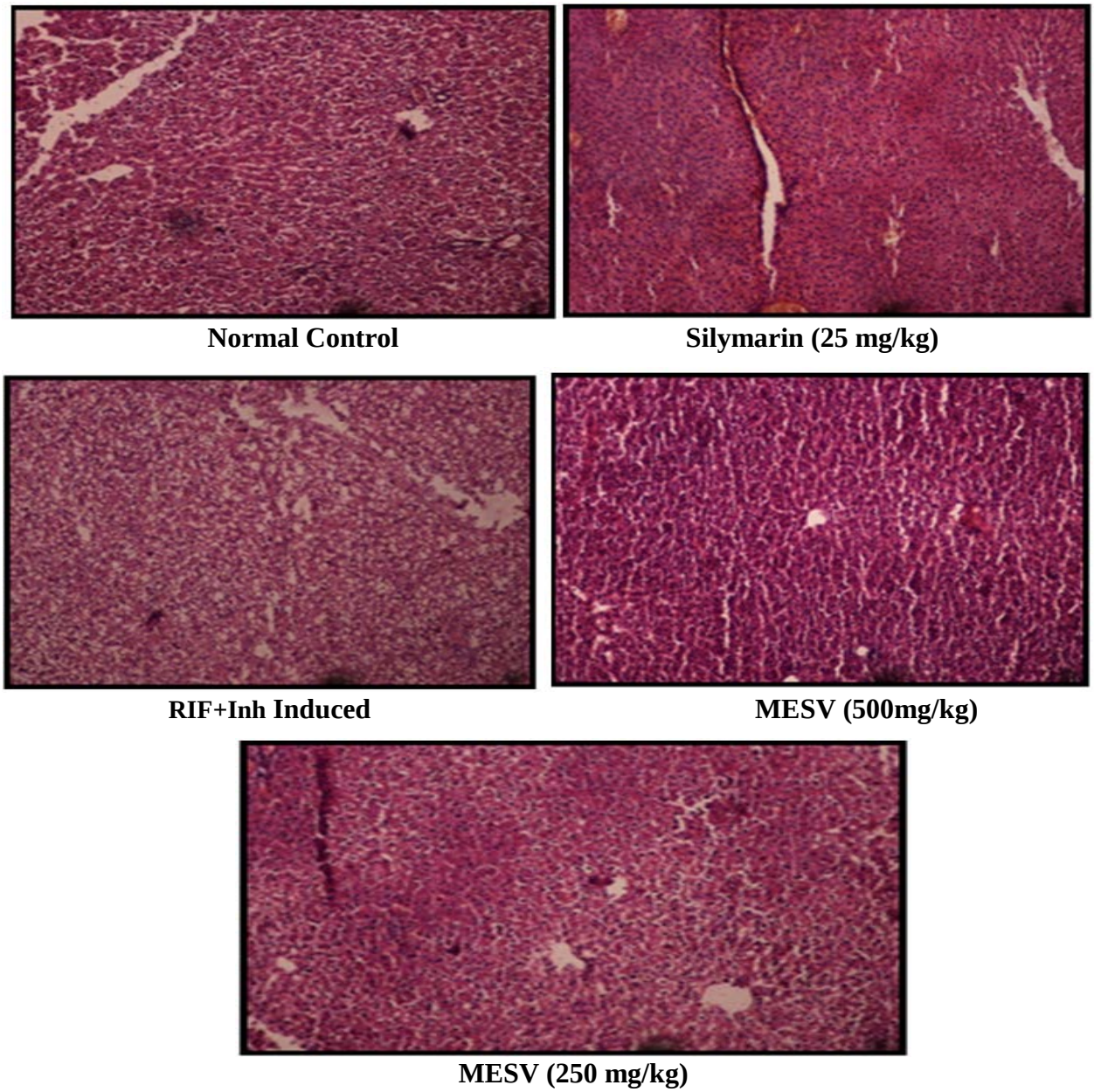

Figure 1: Effect of selected plant extracts on histopathological diagram of liver tissue in RIF+INH induced hepatotoxic rats.

Discussion

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study RIF+INH was used to induce hepatotoxicity, since it is clinically relevant. Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indications of hepatocellular injury **(Yue *et al.*, 2006)**.

In the present study *MESV* at a dose of 250 & 500 mg/kg, *p.o.* caused a significant inhibition in the levels of SGOT and SGPT towards the respective normal range and this is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by RIF+INH. On the other hand suppression of elevated ALP activities with concurrent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants **(Mukherjee *et al.*, 2002).**

These results indicate that *MESV* at a dose of 250 & 500 mg/kg, *p.o.*, *preserved* the structural integrity of the hepatocelluar membrane and liver cell architecture damaged by RIF+INH which was confirmed by histopathological examination.

Phytochemical screening revealed that *MESV* at a dose of 250 & 500 mg/kg, *p.o.,* contains active pharmacological constituents such as flavonoids, alkaloids, phytosterols and phenolic compounds **(Saluja *et al.*, 2011)** However, it has been already reported that such phytoconstituents like phenolic compounds, flavonoids, tannins **(Paya *et al.*, 1993)** are known to possess hepatoprotective activity in various experimental models. Therefore it has been suggest that the hepatoprotective activity shown by the *MESV* at a dose of 259 & 500 mg/kg, *p.o.,* can be because of these active phytoconstituents present in the plant which is being also confirmed by the biochemical and histological parameters. The plant selected for the present study has demands for further phytochemical as well as pharmacological research such isolation of principle active phytoconstituents, evaluation of various pharmacological activities. Out of these aspects some respective parameters are already in process at our organisation.

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