MODULATION OF RIFAMPICIN INDUCED DEPLETION OF GLUTATHIONE IN LIVER OF MALE ALBINO RATS BY MANDUR BHASMA

BAKARE, R. V. AND NALAWADE, S. P.¹

Department of Zoology, Kisan Veer Mahavidyalaya, Wai 412803 (M.S.). ¹Department of Zoology, Yashawantrao Chavan Institute of Science, Satara 415001 (M.S.). E. Mail: ravi_bakare@indiatimes.com

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Abstract: Rifampicin is used to treat tuberculosis. However, the drug is also known to cause hepatotoxicity and exhibit increased incidence of hepatitis. This has been postulated to be due to rifampicin induced cytochrome induction causing an increased production of toxic metabolites. Glutathione is a small molecule made up of three amino acids that is produced in liver and exists in almost every cell of the body. Presence of Glutathione fulfills the need of antioxidants and plays role in biotransformation of drugs. Patients on concurrent rifampicin treatment show depletion of glutathione in liver. The aim of present work is to observe hepatoprotective effect of Mandur Bhasma on the rifampicin generated hepatotoxicity due to depletion of antioxidant.

Male albino rats with 6 animals in each group were divided into four groups for the experiment. Group I was used as control, Group II was treated with rifampicin (50mg/kg body wt/day), Group III was treated with rifampicin (50mg/kg body wt/day) + mandur bhasma (10mg/kg body wt/day) and Group IV was treated with mandur bhasma (10mg/kg body wt/day) for 30 days. Liver and kidney were assayed for the content of glutathione. Restoration of glutathione level is observed when mandur bhasma given with the dose of rifampicin, while the level of glutathione level increased significantly in the liver of rats receiving only mandur bhasma.

Key words: Rifampicin, Mandur Bhasma, Glutathione

INTRODUCTION

Tuberculosis is very common in India and in several European countries including U.S.A. It can be cured by effective antibiotic drug Rifampicin, as it is effective against Mycobacterium tuberculosis. Adverse effects of Rifampicin in patients are reviewed in the [1-4]. Some of the important side effects are hepatotoxicity and renal toxicity. The liver is a versatile organ and exogenous and endogenous nutrients and chemicals (xenobiotics) are absorbed, concentrated, modified and processed called biotransformation. Further it is sent to the gastrointestinal tract or kidney for clearance [3,5,6]. The biotransformed compounds may cause the toxicity rather than the detoxification. The toxicity can be studied by biochemical assay in relation with the glutathione activity in the liver and kidney. The role of N-acetylcysteine (100mg/kg/day for 3 weeks), a glutathione precursor was investigated [7] in protection against rifampicin + isoniazid (each 50mg/kg/day) induced oxidative hepatic injury in young Wistar rats. The oxidative stress was observed, with increased lipid peroxidation and histological lesions that ranged from interlobular inflammation to patchy necrosis. Status of liver protein and energy malnutrition played important role in the pathogenesis of isoniazid + rifampicin (each 50mg/kg/day intraperitonially) induced hepatic injury in weanling rats, growing rats and young rats. Serum transaminases were increased coupled with hepatic
necrosis while hepatic thiols blood glutathione were decreased; similarly antioxidative enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferases were significantly declined while lipid peroxidation was elevated. Oxidative injury was closely associated with significant decline of glutathione and related thiols in hepatoprotection as antioxidative profile. Fatty liver induction was studied by high doses of rifampicin in rats [8].

The reduction in the frequency in the side effects or the toxic effects can improve the place and potency of rifampicin. Therefore, improvement in toxicity problems will give efficacy to rifampicin in its clinical use. This will reduce the dose of rifampicin and the cost of the treatment. Anyhow this requires first animal trials followed by clinical trials.

**MATERIAL AND METHODS**

**Animals:** Male albino rats, obtained from Haffkine Institute, Mumbai, were maintained and bred in departmental animal house (Registered for “Research and Breeder” No.233/CPCSEA) under standard laboratory conditions. The rats were fed standard pellet diet (rat feed) prepared by Amrit feeds (Navmaharashtra Chakan Oil Mills, Sangli, Maharashtra, India) and water ad libitum during breeding, maintenance and experimental work.

**Preparation of Mandur Bhasma:** Mandur bhasma is an Ayurvedic drug, which is already tested as hepatoprotective, and hepatocurative drug. It was also proposed to use to test its influence on Rifampicin induced hepatotoxicity. Mandur bhasma is the ore of the iron in the form of ferric oxide obtained from mines. The drug was purchased from the local suppliers of Ayurvedic medicines and grinded to form very fine dust. It was soaked in the juice of fruits of *Garcinia indica* for twenty-four hours and washed thoroughly with water. 1Kg powder of Mandur was titrated with the five liters of juice of leaves of *Aloe vera* and dried. The bhasma was prepared by giving Gaja puta.

The quality test for Mandur bhasma was conducted and described in the Ayurvedic and Sidha tests. Mandur bhasma was given as per body weight (10 mg/kg/day) for 30 days. A colloidal solution in water was prepared and was given by using feeding tube.

**Rifampicin:** Rifampicin and other derivatives of Rifamycin were considered as relatively non-toxic antibiotics having a broad antibacterial spectrum and are active against *Mycobacterium* species. It is available at medical shops in injectable form, in suspension and powder in encapsulated form. Rifampicin in free powder form was obtained directly from manufacturer- Li-Taka Pharmaceuticals, Pune. We thank respective authorities of the company for supply of free sample. Rifampicin is widely used as anti-tubercular drug throughout the world. However, the drug is also known for the induction of hepatotoxicity.

**Dose of rifampicin:** To study the biochemical alterations occurring in liver, kidney and blood during experimental work healthy male albino rats were given desired dose of Rifampicin (50 mg/kg body wt/day) for 30 days. A paste of drug in water was prepared and was fed by using feeding tube. 24 hours after last dose they were sacrificed and they were tested for desired parameters.

**Experimental protocol:** As mentioned earlier the experiments were conducted in four sets- the rats weighing 130 to 140 g were used for the present experiment. The animals were grouped into 4 groups [each containing 6 animals] and the rifampicin, rifampicin + mandur bhasma and mandur bhasma oral treatments were given daily between 8-00 to 9-00 a.m. Following were the Groups of the animals.

- **Group I - Control:** Normal male albino rats were provided with the normal diet and water *ad libitum* until experimental set was completed.
- **Group II - Rifampicin treated Rats:** To these animals daily dose of 50 mg /kg body wt/day of Rifampicin was given for 30 days.
- **Group III - Rifampicin + mandur bhasma treated Rats.** These rats were fed with 50 mg rifampicin/kg body wt/day and 10 mg mandur bhasma/kg body wt/day for 30 days.
- **Group IV - Mandur bhasma treated Rats:** This group of rats was maintained as Control for Mandur bhasma by giving 10 mg mandur bhasma/kg body wt/day for 30 days.

Immediately after the oral dose, food was supplied to the animals. They were deprived of food for 12 hrs prior to killing. The rats were killed after 24 hr of the last dose given by giving deep ether anesthesia.
Preparation of homogenate: The pieces of liver from central region of central lobe, full transverse sections of kidney were used ensuring the inclusion of medulla and cortex in natural proportion and known quantities of liver, kidney were homogenized in Teflon homogenizer with four strokes at 1300 rpm. The homogenates were diluted with distilled water to a known volume.

Biochemical estimation: Protein was estimate by Lowry et al. technique [9] and glutathione by Grunert and Phillips method [10].

RESULTS

Glutathione content in liver and kidney of rats is given in the table and in the graph. Normal rat liver and kidney exhibited 30.28 ± 2.06 and 32.39 ± 0.231 µM of glutathione /mg protein respectively. Treatment of rifampicin for 30 days caused reduction in glutathione content by 43.53 and 37.14% in rat liver and kidney of group II. Administration of mandur bhasma along with rifampicin elevated glutathione levels in the liver and kidney of group III rat by 02.02 and 01.75 folds in liver and kidney on comparison with the respective values in group II rats treated with rifampicin. The values of glutathione in liver 01.14 fold and kidney 01.10 fold of group II rats were higher than the normal levels. When only mandur bhasma was given to the rats of group IV caused the rises in the glutathione contents in liver and kidney. The elevations of 01.43 and 01.47 folds were noted in liver and kidney of group IV rats as compared to the normal values.

DISCUSSION

In present work 50 mg rifampicin per kg body wt. was administered orally for 30 days. The experimental studies in this project were designed to study the influence of mandur bhasma an Ayurvedic medicine, which is also recommended in Ayurveda to treat the ‘yakshma’ i.e. TB. Mandur bhasma had shown hepatoprotection against CCl4 hepatotoxicity [11] in male albino rats. The protection against CCl4 was studied by the function of different lipases which revealed that CCl4 induced fat accumulation was removed through lysosomal degradation and the fatty acids were used as energy source for tissue

![Graph showing influence of mandur bhasma on glutathione content of Liver and Kidney during rifampicin induced toxicity in albino rats.]

Table 1: Influence of mandur bhasma on glutathione content of liver and kidney during rifampicin induced toxicity in albino rats. Values are expressed in µM/mg protein.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Kidney</th>
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<tbody>
<tr>
<td>Normal</td>
<td>30.28 ± 2.06</td>
<td>32.39 ± 2.31</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>17.1 ± 1.38</td>
<td>20.36 ± 1.52</td>
</tr>
<tr>
<td>Rifampicin, Mandur Bhasma</td>
<td>34.56 ± 1.70</td>
<td>35.55 ± 2.08</td>
</tr>
<tr>
<td>Mandur Bhasma</td>
<td>43.29 ± 2.19</td>
<td>47.59 ± 2.62</td>
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rebuilding. It had been also showed that mandur bhasma is capable of curing induced CC14 hepatotoxicity and liquid paraffin toxicity simultaneously curing the kidney [12] in male albino rats. The studies also revealed that mandur bhasma decreased lipid peroxidation and increased glucose-6-phosphatase activity. For this reason it was decided to use mandur bhasma against the rifampicin-induced hepatotoxicity. Mandur bhasma was administered simultaneously with the rifampicin treatment. The liver and kidney function - tests showed that it also protects liver and kidney against the rifampicin toxicity. The histological architecture also confirmed these results. To study the probable alterations in other toxicity related parameters; the lipid peroxidation, glutathione, formaldehyde and protein oxidation were also studied in liver and kidney. As the results indicate lipid peroxidation, formaldehyde, and protein oxidation were reduced and glutathione was increased in liver and kidney by simultaneous treatment of mandur bhasma in rifampicin treated rats. It seems that liver and kidney are relieved from the oxidative stress by the increased glutathione. It was also increased in the only mandur bhasma treated rats. It seems that Mandur bhasma is stimulating glutathione production. This was also observed in earlier results where mandur bhasma stimulated lysosomal lipid degradation [10].

Though the present study showed protection of liver and kidney by oxidative stress relief and keeps the stress at least in the range that can maintain the histological architecture of liver and kidney. But this work is preliminary and probable mode/s of action can be made out in detail. Still a question remains whether the mandur bhasma will behave the same way in presence of tuberculin infections also? There is need that the curative effects of mandur bhasma be studied that may be helpful to the post-toxicity relief. Work in this respect is in progress.

REFERENCES