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Original Article

HEPATOPROTECTIVE ACTIVITY OF *BAUHINIA VARIEGATA* AGAINST ISONIZED AND RIFAMPICIN-INDUCED TOXICITY IN EXPERIMENTAL RATS

ANIL MARASANI*

Department of Pharmacology St. Peter's Institute of Pharmaceutical Sciences,Vidya nagar, Hanamkonda, Warangal, A.P, INDIA. 506001.

Email: anilmarasani1987@gmail.com

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ABSTRACT

Objective: Besides the great efficacy of isoniazid (INH) and rifampicin (RMP) combination, in the treatment and chemoprophylaxis of tuberculosis, hepatotoxicity is the most common serious complication. The aim of present study is to evaluate the Hepatoprotective activity of Bauhinia variegata against a rat model of INH–RMP induced hepatotoxicity.

Methods: Wistar albino rats of either sex (200-250 g) were treated with alcoholic extract of *Bauhinia variegata (BV)* (200 and 400 mg/kg; p.o) for 15 days. Hepatotoxicity was induced by oral administration of INH (50 mg/kg, p.o.), RMP (100 mg/kg p.o.) body weight /day each. Silymarin (50 mg/kg) used as reference drug. Hepatic marker enzymes, Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP); Gamma glutamyl transferase (GGT), Lactate dehydrogenase (LDH), lipid peroxidation (LPO) in liver and cholesterol (CHO), triglycerides (TGL), Albumin (ALB), Bilirubin and total protein(TOP) in serum was estimated in experimental rats after 15 days.

Results: Levels of marker enzymes (SGOT, SGPT, ALP, GGT, and LDH), ALB, TOP, CHO, and TGL were assessed in serum. The effect of BV on LPO was assayed in liver homogenates to evaluate antioxidant activity. *BV* and Silymarin elicited a significant hepatoprotective activity by lowering the levels of serum marker enzymes and lipid peroxidation.

Conclusion: Histopathological examination revealed preservation of liver integrity of the protected groups compared to combination-treated rats alone. The present findings suggest that the hepatoprotective effect of *BV* in INH-RMP induced oxidative damage may be related to its antioxidant and free radical scavenging activity.

Keywords*:* Isoniazid; Rifampicin; *Bauhinia variegata*; Hepatoprotective; Marker enzymes; Lipid peroxidation.

INTRODUCTION

The liver is the principal organ that is capable of converting drugs into forms that can be readily eliminated from the body. The diversity in drug use/ polypharmacy today and the complex burden they impose upon the liver, it is not surprising that a broad spectrum of adverse drug's effects on liver functions and structures has been observed.

Drug-induced hepatotoxicity, a leading cause of liver injury, poses an important challenge to clinicians. Isoniazid (INH) and rifampicin (RMP), the most important first line antitubercular drugs (ATD) have been used for the treatment of TB. These drugs are also used in combination with other medicines to treat co-infections, and to reduce the duration of anti-TB therapy from 18 months to 6 months [1]. ATD are the most common cause of drug-induced acute liver failure in India [2]. Most of the potent ATD, particularly INH, RMP and pyrazinamide are hepatotoxic [3]. The frequency of hepatotoxicity is increased when these drugs are used in combination.

The hepatotoxicity of INH is thought to be initiated by cytochrome P450 mediated metabolism of INH to acetyl hydrazine and hydrazine [4]. RMP generally co-administered with INH in the treatment of tuberculosis. Its antibacterial activity is mediated by the inhibition of bacterial RNA polymerase. Furthermore, rifampicin is considered as a powerful inducer of mixed-function oxidase that increases the hepatotoxicity of isoniazid by enhancing the production of toxic metabolites from acetylhydrazine [5] and enhances hydrazine production [6] by enzyme induction. The high reactivity of hydrazine with sulfhydryl groups results in glutathione (GSH) depletion within the hepatocytes [4] leading to cell death [7].

The polyphenolics including flavonoids, which are found in many herbal extracts, have been shown to be strong ROS scavengers, antioxidants [8] and protectors of neurons from lethal damage. Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidant chain reactions at small concentrations and pathological processes [9]. Phenolic compounds present in medicinal plants have been reported to have powerful antioxidant activity. Flavonoids are a major class of phenolic compounds present in medicinal plants and are found to have a potential role in prevention of various diseases through their antioxidant activity [10]. *Bauhinia variegata (BV)* Linn. (Caesalpiniaceae) is a medium-sized deciduous tree found throughout India. It is traditionally used in bronchitis, leprosy, and tumours. The stem bark is used as astringent, tonic, and antihelmintic [11]. Infusion of the leaves is used as a laxative and for piles. Dried buds are used in the treatment of worm infestations, tumours, diarrhoea, and piles. The stem bark is used in ayurveda for its antidiabetic activity. The stem bark is reported to contain 5,7 dihydroxy and 5,7 dimethoxy flavanone-4-O-α-L rhamnopyrosyl- ß - D-glycopyranosides, Kaempferol-3-glucoside, lupeol, and beta sitosterol. Since polyphenolic compounds are present in the ethanolic extracts of stem bark of *Bauhinia variegata* Linn. The present experiment was designed to evaluate the alcoholic bark extract of *Bauhinia variegata* for hepatoprotective activity and to study its effect on lipid peroxidation and activities of the associated hepatic marker enzymes Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP); Gamma glutamyl transferase (GGT), Lactate dehydrogenase (LDH) during INH - RMP induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Wistar strain albino rats of either sex weighing 200–250 g were used for this study. Animals were housed in cages at an ambient

temperature of 25±2°C and 45–55% relative humidity with 12 h light/dark cycle. They had free access to standard pellet chow (Brook Bond, Lipton India) and water *ad libitum*. Animals were divided in to 5 groups of six animals each. The experimentations on animals were approved by the Institutional Animal Ethical Committee (IAEC) under the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. Approval No: 1018/SPIPS/Wgl/IAEC/2010.

Chemicals

Thiobarbituric acid (HiMedia Laboratories Ltd., Mumbai), Trichloroacetic acid (Qualigens Fine Chemicals, Mumbai), 1,1,3,3,-Tetraethoxy propane (Sigma, St. Louis, USA), Isoniazid and Rifampicin (Gift samples from Dr. Reddys, Hyderabad), Silymarin (Gift sample from Medrich, Bangalore), SGOT, SGPT, ALP, GGT, ALB, Bilirubin, TGL, CHO, and TOP auto analyser kits from Coral clinical bio systems, Hyderabad. The other chemicals and solvents used were of analytical grade purchased from commercial suppliers.

Extraction of the Plant Material

The stem bark of *BV* Linn was collected from the Botanical Garden, and authenticated from the Dept. of Botany, Kakatiya University (KU). Plant Specimen (voucher no: KUH 1854) was submitted in the Herbarium, Dept. of Botany, KU. Bark was dried in shade and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvent, 95% alcohol. Course powder (240 gm) was soxhlet extracted with 95% alcohol (2450 ml). The resultant alcoholic extract was concentrated by rotary vacuum evaporator. The extracts were then freeze-dried and stored in a vacuum desiccator (yield 25%, w/w). The extract was stored in an airtight container in a cool place and used throughout the project.

Acute Toxicity Study and Gross Behaviour in Rats

Acute toxicity study – up and down procedure – was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD). If animal dies at particular dose, lower dose is given to the next animal and if animal survives at a particular dose, next higher dose was given for remaining animals. The maximum upper limit dose 2000 mg/kg of *BV* was administered orally to mice. Animals were observed individually after dosing. Observation included mortality and clinical signs, such as changes in skin fur, eyes and mucous membranes. The gross behaviours, e.g. body positions, locomotion, rearing, tremors, gait was observed. The effect of BV on passivity, grip strength, pain response, stereotypy, vocalization, righting reflex, body weight and water intake was assessed [12]. Pilot study was carried out with various doses (50, 100, 200 and 400 mg/kg, per oral route to rats) of BV. At doses of 200 and 400 mg/kg, it was active and at 50 mg/kg it was inactive. Based on this observations two different doses (200 and 400 mg/kg) of BV was selected in INH-RMP induced hepatotoxicity model.

Drug Treatment Protocol

Animal are divided into 5 groups of 6 animals each. Normal control group (Group I) received only vehicle (5% acacia solution) without hepatotoxicity, whereas animals from model control group (Group II) received only INH-RMP without any treatment. Animals from Group III to Group V received test drugs such as standard drug Silymarin (50 mg/kg p.o.), and alcoholic extract of *BV*, (200 mg/kg; 400 mg/kg p.o.), once daily for 15 days 45min prior to INH-RMP treatment.

Isoniazid, Rifampicin induced hepatic damage

Hepatic damage was induced by administration of INH (50 mg/kg); RMP (100 mg/kg) orally, once daily induced hepatic injury. Drugs were given as suspension orally 45 min before standard and test drugs treatment. The animals were killed on 15th day after the dose of INH–RMP administration. Rats had free access to food and drinking water during the study.

Biochemical estimations

Biochemical tests to estimate lipid peroxidation in liver by Okawa et al., method [13], SGOT& SGPT by Mod. IFCC method, ALP by Mod. Kind & King's method [14], GGT by Carboxy substrate method, albumin by BCG method [15]. bilirubin by Mod. Jendrassik and Grof's method [16], triglycerides by GPO/PAP method [17], cholesterol by CHOD/PAP method [17], and total protein by Biuret method [18], LDH by Mod. IFCC method was performed using standard procedures reported in the literature.

Histopathology studies

At the end of the treatment, rats were sacrificed by cervical decapitation. Livers were excised, washed in physiological saline to remove blood clot and other tissue materials, and fixed in 10% formalin saline. Sections were prepared and stained with hematoxylin and eosin for the histological investigations.

Statistical analysis

Results were expressed as mean±S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found statistically significant ($P < 0.05$), further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses and the diagrammatic representation of the data were performed by using Graph pad PRISM, Version 5 software.

RESULTS

Effect of BV in acute toxicity and gross behaviours in rats

We found that there was no mortality up to 2000 mg/kg dose. The rats treated with *BV* at the dose of 2000 mg/kg were well tolerated and exhibited normal behaviour. Rats were alert with normal grooming, touch response, pain response and there was no sign of passivity, stereotypy, and vocalization. There was no abnormal change in motor activity, secretory signs as well as their body weight and water intake.

Effect of BV in INH, RMP induced hepatotoxicity

Increase in liver weight (P< 0.001) was observed in hepatotoxicity rats compared to normal (Table 1).

Table 1: Protective effect of ethanolic extract of *Bauhinia variegata* (*BV*) on Liver weight in rats subjected to INH-RMP induced hepatotoxicity

Group	Liver weight (g/100 g)
Control (vehicle)	2.36±0.33
INH-RMP induced hepatotoxicity (HT)	3.65±0.06[a]
HT +Silymarin (50 mg/kg)	2.45±0.04[b]
HT +*BV*200 mg/kg	3.08±0.06[a,b]
HT +*BV*400 mg/kg	2.35±0.04[b]

All the data were expressed as mean ± SEM, n = 6. a = p< 0.001 vs control; b = p< 0.001 vs INH-RMP induced hepatotoxicity.

INH-RMP treated group of animals showed a significant increase (P< 0.001) in the levels of the marker enzymes and a decrease in ALB and TOP as compared with the normal group. Treatment with *BV* (200 mg/kg, 400 mg/kg) as well as Silymarin (50 mg/kg) decreased the elevated levels of SGOT, SGPT, GGT (Figure 1), ALP, LDH (Figure 2) TGL, CHO (Figure 3), and Bilirubin by INH-RMP and increased the ALB and TOP levels (Table 2).

Increased LPO was observed in INH-RMP induced hepatotoxicity group compared to the normal control group. *BV* treatment significantly reverted back the elevated levels of LPO to normal.

After assessing the liver histology, control group showed a normal morphology in all animals. In INH–RMP treated group, of animals showed moderate to heavy lobular inflammation, hepatocytes

degradation and necrosis. Treatment with Silymarin showed recovery from toxicity. Treatment with *BV* showed amelioration of liver injury (Fig. 4).

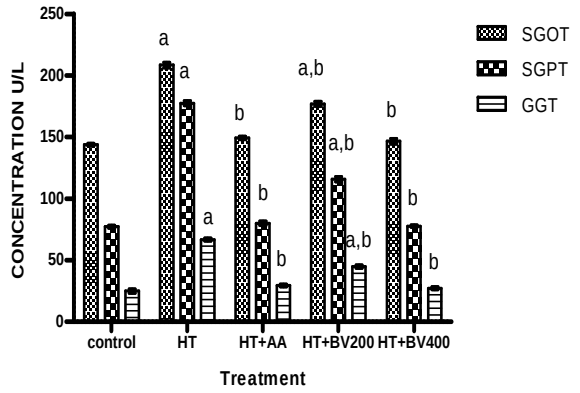

Fig.1: Protective effect of ethanolic extract of *Bauhinia variegata* (*BV*) on SGOT, SGPT, GGT levels in rats subjected to INH- RMP induced hepatotoxicity (HT). All the data were expressed as mean ± SEM, n = 6. a = p< 0.001vs control; b = p< 0.001vs HT.

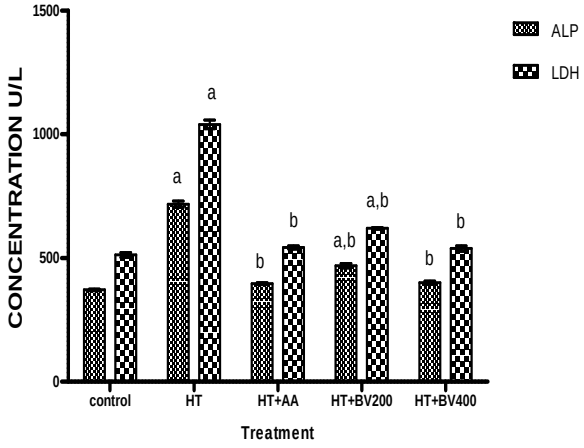

Fig.2: Protective effect of ethanolic extract of *Bauhinia variegata* (*BV*) on ALP, LDH levels in rats subjected to INH- RMP induced hepatotoxicity (HT). All the data were expressed as mean ± SEM, n = 6. a = p< 0.001vs control; b = p< 0.001vs HT.

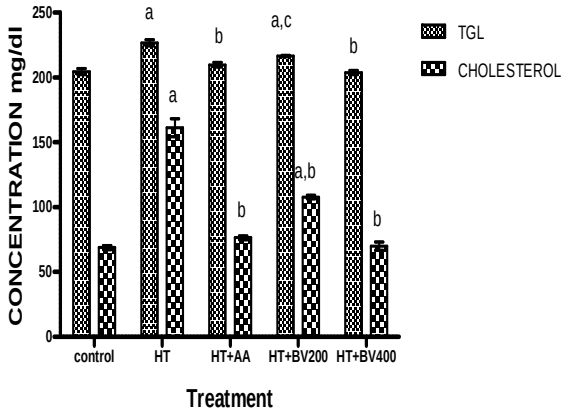

Fig.3: Protective effect of ethanolic extract of *Bauhinia variegata* (*BV*) on Cholesterol and Triglyceride levels in rats subjected to INH- RMP induced hepatotoxicity (HT). All the data were expressed as mean ± SEM, n = 6. a = p< 0.001vs control; b = p< 0.001 vs HT.

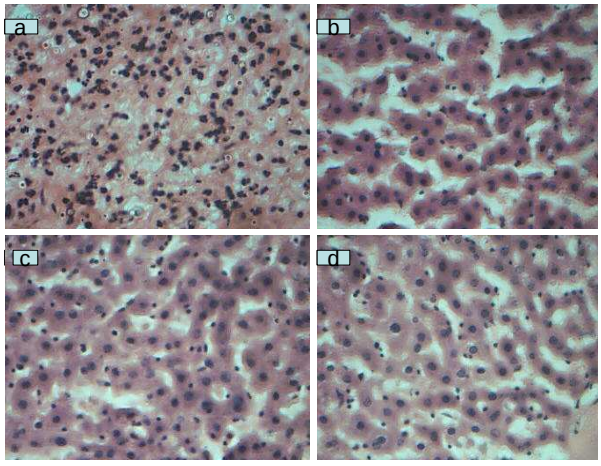

Fig.4: a) Photograph of normal rat liver (H&E 400x). b) Photograph of rat liver treated with INH-RMP (H&E 400x) showing necrosis and hepatocytes degradation. c) Photograph of rat liver treated with INH-RMP and Silymarin (H&E 400x) showing mild degradative effect (almost complete recovery, normal hepatocyte morphology). d) Photograph of rat liver treated with INH-RMP and *Bauhinia variegata* (H&E 400x) showing few degraded cells (signs of amelioration of INH-RMP induced liver injury i.e less degree of necrosis, mild fatty change).

Table 2: Protective effect of ethanolic extract of *Bauhinia variegate* (*BV*) on Bilirubin, Albumin, Total protein, and LPO levels in rats subjected to INH-RMP induced hepatotoxicity

Group	Bilirubin (mg/dl)	Albumin (g/dl)	Total protein (g/dl)	MDA (nmol/g protein)
Control (vehicle)	0.272±0.021	4.53±0.128	7.4±012	3.58±0.079
INH-RMP induced hepatotoxicity (HT)	0.558±0.024[a]	3.48±0.087[a]	4.8±0.136[a]	7.2±0.208[a]
HT +Silymarin (50 mg/kg)	0.325±0.04[d]	4.32±0.087[d]	6.98±0.125[d]	3.93±0.061[d]
HT +*BV*200 mg/kg	0.39±0.015[b,d]	4.0±0.058[a,e]	5.58±0.122[c,e]	5.68±0.065[a,d]
HT +*BV*400 mg/kg	0.295±0.013[d]	4.45±0.089[d]	7.25±0.106[d]	3.93±0.120[d]

All the data were expressed as mean ± SEM, n = 6. a = p< 0.001, b = p< 0.05, c = p< 0.01 vs control; d = p< 0.001, e = p<0.01 vs Iron overload.

DISCUSSION

Administration of INH–RMP combination produces many metabolic and detoxifying sites for these antitubercular drugs [19]. The current study was conducted to investigate firstly, the involvement of oxidant–antioxidant balance in the hepatotoxicity induced by the combined INH–RMP administration; INH–RMP administration significantly increased SGOT, SGPT, ALP, GGT, LDH, and Bilirubin

activities together with a significant decrease in serum protein, albumin levels. This can be attributed to hepatic structural damage as these enzymes normally localized in the cytoplasm and released into the circulation after cellular damage has occurred [20]. Such hepatotoxic effect induced by INH–RMP administration was confirmed by histopathological findings. These observations were faithfully supported by a paralleled improvement in histopathological examinations.

Pretreatment with *BV* (at both the doses 200 and 400 mg/kg) as well as the reference drug Silymarin, the levels of these marker enzymes in liver were nearly normal or only slightly elevated, indicating protection against liver damage. *BV* has increased the levels of total proteins and albumin in the serum, which indicates hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells.

Consumption of isoniazid and rifampicin increased the bilirubin level in the serum of hepatotoxic rats. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release [21]. Co-administration of *BV* restored the level of bilirubin to near normal status by its cytoprotective and may be also due to the inhibitory effect on cytochrome P_{450}. Isoniazid and rifampicin induced hepatitis is due to their biotransformation to reactive metabolites that are capable of binding to cellular macromolecules [22]. As an alternative to inducing cellular damage by covalent binding, there is evidence that these antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defence system. The role of oxidative stress in the mechanism of isoniazid and rifampicin-induced hepatitis has been reported by Attri et al., 2000 [23]. Our findings confirm the same pattern and show significant increase in the level of lipid peroxidation in liver tissue of antitubercular drugs administered rats as compared to that of control rats. In the present study, free radicals formed either by the reaction of metabolites of INH/RMP with oxygen or by the interaction of superoxide radicals with H_2O_2, seem to initiate peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to formation of lipid peroxides which in turn give products like MDA that cause loss of integrity of cell membrane and damage to hepatic tissue. In rats treated with INH-RMP alone, the increase in TBARS indicates enhanced lipid peroxidation leading to a failure of the antioxidant defence mechanism to prevent formation of excess free radicals [24]. Pretreatment with *BV* prevented significantly lipid peroxidation either directly or through GSH by scavenging the free radicals.

The major disorder encountered in antitubercular drugs-induced hepatotoxicity is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition. In the present study, total cholesterol, and triglyceride levels were significantly elevated in INH-RMP combination treated rats. The beneficial hypolipidemic effects of *BV* are in agreement with the results obtained by other investigators [25], where *BV* supplementation was effective against Triton WR-1339 induced dyslipidemia [26]. The mechanism by which BV is able to reduce total cholesterol and Triglycerides concentration is not clear, but probably via modulation of lipoprotein lipase activity or cholesterol metabolism by the liver [27]. This present study indicated marked increase in liver triglycerides content in INH–RIF treated animals. There may be many hypothesise to explain the accumulation of triglycerides in liver of INH–RMP treated rats. First reason may be that INH–RIF treatment showed decrease of lipoprotein synthesis, resulting in impaired mobilization of lipids from the liver.

We observed significantly higher liver and serum cholesterol levels in the animals that received long term INH–RMP treatment compared to those who did not receive the treatment. Previous studies have also shown that INH–RMP treatment lead to inhibition of bile secretion resulting cholesterol accumulation in liver [28]. Normally, cholesterol gets secreted as bile acid in liver. Therefore, similar mechanism may be responsible for the increased cholesterol accumulation in liver in the present study. Other authors have also observed increased plasma cholesterol in hepatotoxicity conditions [29] and in chronic liver diseases [30].

BV stem bark contains flavonoids such as quercetin, flavonone glycosides such as 5,7 dihydroxy [31] and 5,7 dimethoxy flavanone-4-O- α -L rhamnopyrosyl- ß -D-glycopyranosides [32,33] flavonol glycosides characterised as kaempferol-3-glucoside [34], stigmasterol [35] and phenolic compounds, and these pharmacophores have been shown to possess potent antioxidant and free radicals scavenging activity.

The present investigation showed the hepatoprotective potential of alcoholic extract of *BV* against INH-RMP induced oxidative stress. It was observed that *BV* attenuated the impaired liver marker enzymes status in hepatotoxic rats. The activity of *BV* appears to restore the altered marker/antioxidant enzymes as well as decrease the production of LPO in liver induced by INH-RMP combination. This may be due to direct scavenging effect of biflavone on free radical released during hepatotoxicity and also modulate the endogenous antioxidants enzymes mediated hepatoprotection.

CONCLUSION

In conclusion, the two major mechanisms responsible for the hepatoprotective activity of BV are scavenging of ROS and protection against elevated levels of lipid peroxidation. Flavonoids of *B.variegata* are easily absorbed and stabilize the ROS by reacting with them and getting oxidized in turn to more stable less reactive radicals. Presumably, the high reactivity of OH group of flavonoids is responsible for this free radical scavenging activity.

These hepatoprotective effects are positively correlated to their ability to inhibit combination-induced NO overproduction, suppression of neutrophil infiltration and the maintenance of the enzymatic and non-enzymatic antioxidant balance. Furthermore, BV exerts an additional anti-lipidemic property against combination-induced hepatotoxicity. The antitubercular drugs (isoniazid and rifampicin)-induced alterations on protein metabolism and hepatic antioxidant defence system were normalized by *BV* co-administration, indicating a possible cytoprotective role of *BV* against drug induced hepatitis. Thus BV can be classified as an antihepatotoxic agent.

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