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Preventive and curative effects of Vedic Guard against anti-tubercular drugs induced hepatic damage in rats

Rema Razdan*, Imranulla and Amar Dev M.J.

** Dept. of Pharmacology, Al Ameen College of Pharmacy, Lalbagh road, Bangalore – 560027, India.*
Visveswarapura Institute of Pharmaceutical Sciences, Banashankari 2 nd stage, Bangalore – 560070, India.
***Author for Correspondence** : +91-9886034280, rrazdan2002@yahoo.com*

ABSTRACT

Vedic Guard, a polyherbal formulation in Ayurveda has been investigated for its possible or claimed hepatoprotective potential. Anti-tuberculosis drugs caused an increase in the activity of serum diagnostic markers ASAT, ALAT, ALP and total bilirubin, and a decrease in total plasma protein. Hepatic liver peroxidation was enhanced and free radical scavenging enzyme levels of superoxide dismuase (SOD), catalase (CAT) and glutathione (GSH) were reduced. Administration of Vedic Guard simultaneously with anti-TB drugs for 45 days prevented the alterations induced by the anti-TB drugs in the markers of liver function test and markers of oxidative stress. Treatment with Vedic Guard for 20 days post 45 days administration of anti TB drugs reversed the damage caused by the toxicant. Improvement in the histoarchitecture of liver also supported the biochemical studies. In conclusion, Vedic Guard exhibited preventive and curative effects on the hepatotoxicity induced by anti-TB drugs in rats.

KEYWORDS: Vedic Guard; Hepatoprotective activity; Antioxidative activity.

INTRODUCTION

The liver is an important organ concerned with the biochemical functions in the human body. It detoxifies and synthesizes substances; therefore damage to the liver caused by hepatotoxic agents is of serious matter. There is an ever-increasing need of an agent, which could protect it from such damage. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systemic research methodology and to scientifically evaluate the basis for traditional herbal medicines, which are claimed to possess hepatoprotective activity (1).

Hepatotoxicity is a potentially serious adverse effect of currently used anti-tubercular chemotherapeutic regimens containing isoniazid, rifampicin, and pyrazinamide (2-5). Adverse effects of anti-tubercular therapy are sometimes potentiated by multiple drug regimens. Isoniazid, rifampicin and pyrazinamide are each potentially hepatotoxic, thus when administered in combination their toxic effect is at least additional (6-8). Isoniazid causes liver damage due to its reactive metabolites generated from acetylhydrazine. Rifampicin is an enzyme inducer and enhances formation of reactive metabolites which impair the uptake of bilirubin and cause acute cellular necrosis (15). Pyrazinamide also has the potential to produce hepatocellular damage (16). An increased level of ASAT, ALAT and ALP in serum of anti-TB drug treated rats is due to leakage of these enzymes from liver as a result of tissue damage (6).

Herbal drugs play a major role in the treatment of hepatic disorders (9). A number of medicinal plants and their formulations are used to cure hepatic disorders in the Indian traditional system of medicine (10-12). Vedic guard is a polyherbal formulation containing 16 extracts of well known plants in ayurveda possessing natural antioxidant activity which protects cells from degenerative changes (Table 1). It is used in the treatment of hypertension, diabetes, stress and strain conditions, cardio-vascular diseases, arthritis, digestive and metabolic disorders, cataract and as a co-therapy in the management of tuberculosis, cancer, AIDS and liver cirrhosis. The present study was carried out to explore the effect of Vedic Guard on biochemical and histopathological changes associated with anti TB drugs induced liver damage in rats. As hepatotoxicity induced by anti-TB drugs is found to be mediated through oxidative stress and free radical damage to hepatocytes, the antioxidant property of the polyherbal formulation was also studied.

MATERIALS AND METHODS

Drugs and chemicals **-** The polyherbal formulation

Vedic guard was obtained solely for research purposes from Vedic Biolabs, Bangalore, India. Vedic guard contains extracts of 16 medicinal plants (Table 1). The dried powder of Vedic guard was suspended in 2% w/v gum acacia and used for the in vivo study. Isoniazid, rifampicin and pyrazinamide were supplied by Shubchem Chemicals Pvt, Ltd, Mumbai. Assay kits for serum ASAT and ALAT were purchased from DiaSys Diagnostic System, Germany. Total Bilirubin kit, ALP kit and Total protein kit were purchased from Merck Ltd, Mumbai and Span Diagnostic Ltd, Surat respectively. All the other chemicals were of analytical grade.

Animals

Wistar rats weighing 150-250 g were procured from the Central Animal Research facilities.. The animals were kept under standard laboratory conditions and fed on pellet diet and water ad libitum. The protocol was approved by the Institutional Animal Ethical Committee constituted for the purpose.

Experimental

Wistar rats were divided into 7 groups of 6 animals each. The treatment with isoniazid, rifampicin, and pyrazinamide was carried out for 45 days and the effect of Vedic guard during treatment and after treatment was studied.

The doses of anti-tubercular drugs (Isoniazid [H]-27 mg/kg/day, rifampicin [R]-54 mg/kg/day and pyrazinamide [Z]-235 mg/kg/day) were extrapolated from daily human dose using the conversion table based on body surface area (13). According to the conversion table, the human adult dose multiplied by 0.018 gives the dose for a rat weighing 200g. This was taken as the lower dose and higher dose of 10 times the lower dose were selected for the study (13).

Group 1 rats served as vehicle control group and were administered 2% w/v gum acacia orally for 45 days. Group 2 rats served as control and were administered (H+R+Z) suspension orally for 45 days. Group 3 rats were administered (H+R+Z) suspension and Vedic guard suspension **90** mg/kg/day orally for 45 days. Group 4 rats were administered (H+R+Z) suspension and Vedic guard suspension **900** mg/kg/day orally for 45 days. Group 5 rats were administered (H+R+Z) suspension orally for 45 days and no treatment was given from day 45 to day 65. Group 6 rats were administered (H+R+Z) suspension orally for 45 days and Vedic guard 90 mg/kg/day suspension from day 45 to day 65. Group 7 rats were administered (H+R+Z) suspension orally for 45 days and Vedic guard 900 mg/kg/day suspension from day 45 to day 65.

Assessment of hepatoprotective activity –

At the end of the 45th day, 55th day and 65th day blood was collected from all the groups and analyzed for serum aspartate aminotransferase (ASAT), alanine aminotrasferase (ALAT), alkaline phosphatase (ALP), total bilirubin (TB) and total proteins (TP) by using commercial kits.

Assessment of antioxidants

The animals were sacrificed and the abdomen was cut open to remove the liver. The isolated liver was divided into two parts, in order to prepare liver homogenate as follows: From the first part, 5% w/v homogenate was prepared using cold 0.15M potassium chloride and centrifuged at 800 xg for 20 minutes. The supernatant was used for estimation of lipid peroxidation (LP) in terms of thiobarbituric acid reactive substances (TBARS), and catalase (CAT). From the second part, 5% w/v liver homogenate was prepared using 0.25% w/v sucrose in phosphate buffer, centrifuged at 800 xg for 10 minutes. The supernatant was used for estimation of superoxide dismutase (SOD) and glutathione (GSH).

Histopathological studies

The liver specimens obtained from all groups of animals were fixed in 10% buffered formalin and stained with haematoxylin-eosin for photomicroscopic observations of the liver histological architecture.

Statistical analysis

Results are expressed as mean $\pm$ SEM for determination of significant inter group differences, each parameter was analyzed separately and one-way analysis of variance was carried out. After individual comparison of the groups, mean values were done using Dunnet's test (14).

RESULTS

Animals administered anti-TB drugs for 45 days showed a significant fall in total protein level and a rise in the levels of total bilirubin, ALT, AST and ALP in comparison with the control group. Co administration of Vedic Guard along with the anti-TB drugs (90 mg/kg and 900 mg/kg) significantly ($p<0.01$) minimized these changes.

Withdrawal of anti-TB drugs failed to produce significant reversal of the biochemical parameters within 10 days (group 5, 55th day). Treatment with Vedic Guard (90mg/kg, 900mg/kg) for 10 days after withdrawal of anti-TB drug therapy (group 6, 7, 55th day) significantly reversed ALAT, ALP and total bilirubin levels and failed to reverse ASAT and total protein as compared to group 1. Treatment with Vedic Guard (90mg/kg, 900mg/kg) for 20 days (group 6, 7,

Table 1: Medicinal plant ingredients of Vedic guard.

Botanical name	Common name	Family	Part used
Piper longum	Pippali	Piperaceae	Fruit
Mesua ferrea	Nagakesara	Clusiaceae	Fruit
Eclipta alba	Bhringaraj	Asteraceae	Whole plant
Withania somnifera	Ashwagandha	Solanaceae	Root
Tribulus terrestris	Gokshura	Zygophllaceae	Seeds
Sida cordifolia Linn	Bala	Malvaceae	Whole plant
Tinosporia cordifolia	Guduchi	Meninspermaceae	Stem
Glycyrrhiza glabra Linn	Yastimadhu	Leguminaceae	Root
Terminalia chebula	Abaya	Combretaceae	Bark
Commiphora mukul	Guggulu	Burseraceae	Whole plant
Curcuma longa	Haridra	Zingibraceae	Rhizome
Terminalia arjuna	Arjuna	Combretaceae	Bark
Emblica officinalis	Amalaki	Euphorbiaceae	Fruit
Bacopa monnieri	Brahmi	Scrophulariaceae	Whole plant
Puereria tuberosa	Vidari	Fabaceae	Tubers
Asphaltum	Shilajith		Exudate

Table 2 : ***Effect of Vedic guard on liver function tests in anti-tubercular drugs-treated rats****.*

Group	*S.AST (U/l)*	S.ALT (U/l)	S.ALP (IU/min)	S.TB (mg/dl)	S.TP (g/dl)
Group I	93.05 ± 3.5	27.3 ± 0.6	101.4 ± 2.8	0.15 ± 0.003	6.8 ± 0.1
Group II	144.4 ± 4.4$^{\#\#}$	53.1 ± 2.0$^{\#\#}$	170.09 ± 5.6$^{\#\#}$	0.55 ± 0.05$^{\#\#}$	5.6 ± 0.2 $^{\#\#}$
Group III	94.2 ± 6.8**	24.6 ± 3.6**	89.7 ± 3.6**	0.36 ± 0.04**	6.21 ± 0.02
Group IV	90.9 ± 6.4**	24.8 ± 0.8	93.9 ± 3.2**	0.27 ± 0.01**	6.6 ± 0.1**
Group V 55th Day	145.0 ± 2.0	59.2 ± 1.7	154.6 ± 3.0	0.56 ± 0.04	5.3 ± 0.1
Group VI55th Day	136.1 ± 2.5	47.0 ± 1.6 $^{@@}$	118.5 ± 1.9$^{**@@}$	0.27 ± 0.01$^{**@@}$	6.2 ± 0.2$^{@}$
Group VII 55th Day	130.7 ± 3.9	39.6 ± 1.0 $^{**@@}$	84.5 ± 1.6$^{**@@}$	0.25 ± 0.01$^{**@@}$	6.8 ± 0.1$^{**@@}$
Group V 65th Day	101.5± 4.4$^{**@@}$	54.0 ± 2.6	123.6 ± 2.9$^{**@@}$	0.28 ± 0.03$^{**@@}$	5.7 ± 0.3
Group VI 65th Day	94.7 ± 4.9 $^{**@@}$	30.1 ± 1.2$^{**@@}$	105.2 ± 3.9 $^{**@@}$	0.23 ± 0.02$^{**@@}$	6.7 ± 0.2$^{**@@}$
Group VII 65th Day	96.7 ± 4.7$^{**@@}$	27.2 ± 0.5$^{**@@}$	102.3 ± 9.2 $^{**@@}$	0.18 ± 0.02$^{**@@}$	6.9 ± 0.1$^{**@@}$

All values are mean ± SEM, n= 6 ; Dunnett's test:- $^{\#}$-group II Vs group I. ; group II Vs group III, group IV, group V 55th day, group VI 55th day, group VII 55th day and group V 65th day, group VI 65th day, group VII 65th day. ; $^{@}$-group V Vs group VI 55th day, group VII 55th day and group V 65th day, group VI 65th day, group VII 65th day. ; $^{\#\,@}$ p<0.05; $^{**\#\#\,@@}$ p<0.01;when compared as above.

Table 3 : Effect of Vedic guard on the antioxidant enzymes level in rat liver.

Group	SOD U/100mg tissue	CATALASE U/100mg tissue	TBARS nmoles/100mg tissue	GLUTATHIONE nmoles/100mg tissue
Group I	10.24 ± 1.24	23.86 ± 1.50	2.17 ± 0.25	0.386 ± 0.03
Group II	4.11 ± 0.22$^{\#\#}$	12.87 ± 0.92$^{\#\#}$	7.38 ± 0.79$^{\#\#}$	0.133 ± 0.01$^{\#\#}$
Group III	7.30 ± 0.67	18.27 ± 1.26 *	5.89 ± 0.56	0.354 ± 0.03 **
Group VI	9.37 ± 0.68 **	20.18 ± 0.98**	2.48 ± 0.15 **	0.284 ± 0.03**
Group V	5.96 ± 0.74	15.77 ± 0.79	7.53 ± 0.68	0.140 ± 0.009
Group VI	10.87 ± 1.3 $^{**@}$	23.26 ± 1.2$^{**@@}$	3.41 ± 0.44$^{**@@}$	0.29 ± 0.02 $^{**@@}$
Group VII	10.66 ± 0.84 $^{**@@}$	23.23 ± 1.64 $^{** @@}$	2.46 ± 0.44 $^{**@@}$	0.33 ± 0.02

All values are mean ± SEM, n = 6; Dunnett's test: - $^{\#}$-group II Vs group I. ; *-group II Vs group III, group IV, group V, group VI, group VII. ; $^{@}$-group V Vs group VI, group VII. ; $^{*\#@}$ p<0.05; $^{**\#\#@@}$ p<0.01; when compared as above.

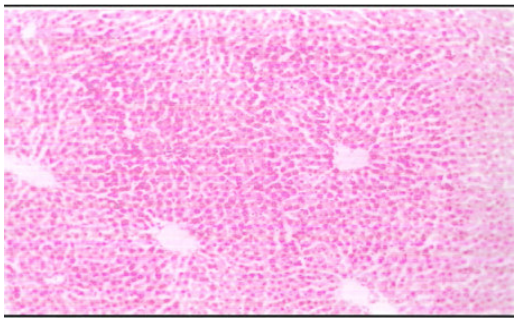

Fig 1: Section of liver from negative control rats (Group1) showing normal appearance of hepatic parenchyma (H & E X 125).

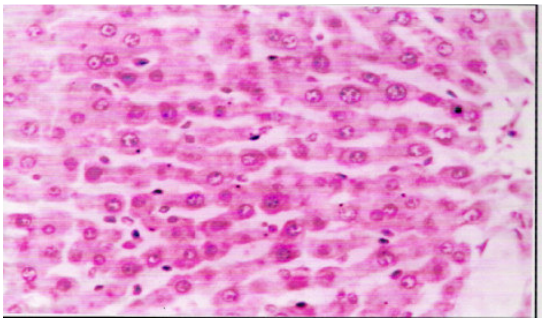

***Fig 2**: Section of liver from negative control rats (Group 1) showing normal appearance of hepatic parenchyma including portal areas (H & E X 500).*

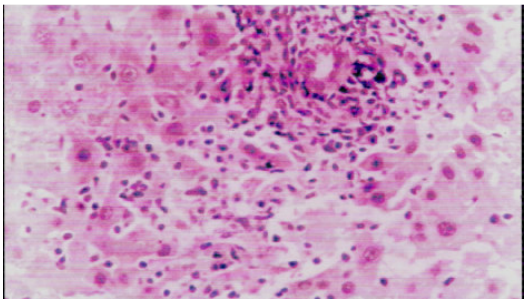

Fig 3: Section of liver from positive control rats (Group 2) showing focal areas of necrosis of few hepatocytes along with portal infiltration of lymphoid cells (H & E X 500).

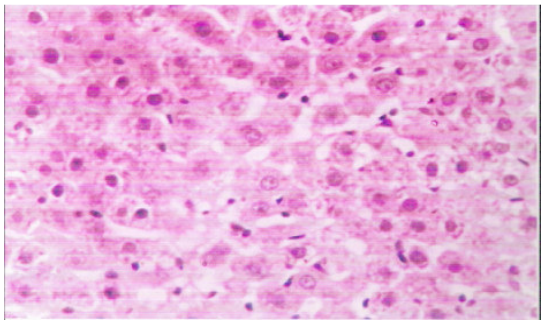

Fig 4: Section of liver from positive control rats (Group 2) showing cell swelling, reduced sinusoidal space, vacuolation of hepatocytes and occasional hepatocyte degeneration (H & E X 500).

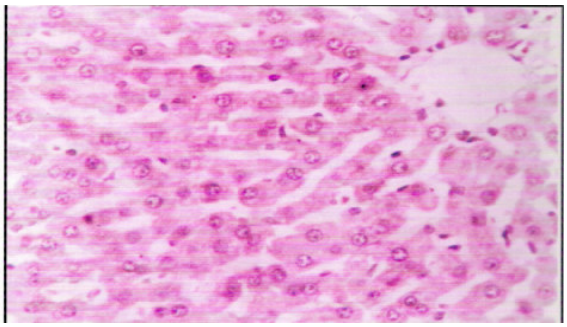

Fig 5: Section of liver from rats treated simultaneously with Vedic guard (90 mg/kg) and (I + R + Z) for 45 days showing normal hepatic parenchyma including the portal areas (H & E X 500).

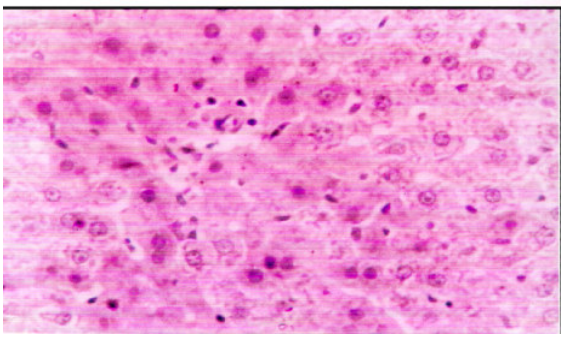

Fig 6: Section of liver from rats treated with (I + R + Z) for 45 days followed by recovery period of 20 days showing pathological changes like cell swelling, reduced sinusoidal space and cytoplasmic vacuolation of occasional hepatocytes (H & D X 500).

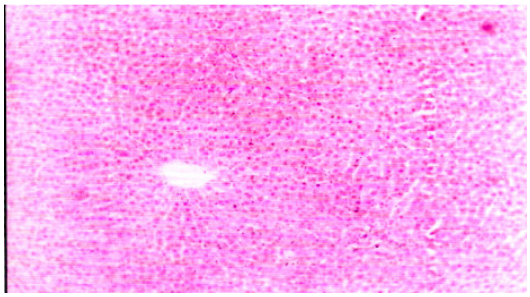

Fig 7: Section of liver from rats treated with (I + R + Z) for 45 days and followed by Vedic guard 90 mg/kg from day 45 to day 65 showing normal hepatic parenchyma (H & E X 125).

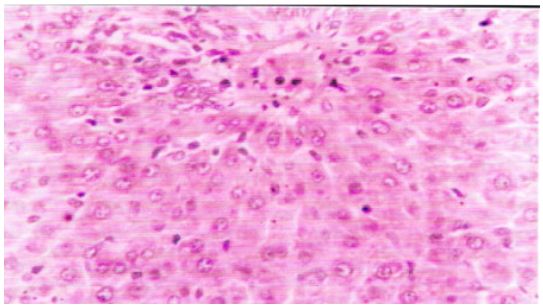

Fig 8: Section of liver from rats treated with (I + R + Z) for 45 days and followed by Vedic guard 90 mg/kg from day 45 to day 65 showing normal hepatic parenchyma including portal areas (H & E X 500).

65th day) after withdrawal of anti-TB drug therapy significantly ($p<0.01$) reversed all biochemical changes as compared to group 2; the reversal was significant when compared with the values for group 5.

The antioxidant liver enzyme levels of group 2 showed a significant decrease in catalase, SOD and glutathione, while a significant increase in TBARS was observed when compared to normal rats. Co administration of lower dose of Vedic Guard to group 3 animals significantly prevented the anti-TB drugs induced decrease in the levels of catalase ($p< 0.05$) and glutathione ($p< 0.01$) but failed to prevent changes in SOD and TBARS levels .Co administration of higher dose of Vedic Guard to group 4 significantly ($p< 0.01$) prevented the anti-TB drugs induced decrease in the levels of SOD, catalase and glutathione and increase in TBARS.

Withdrawal of anti-TB drugs failed to produce significant reversal of any of the antioxidant liver enzyme levels in 20 days (group 5, 65th day) as compared to group 1 Treatment with Vedic Guard (90 mg/kg, 900mg/kg) for 20 days after withdrawal of anti-TB drug therapy significantly (0.01) reversed the anti-TB drug induced decrease in the levels of SOD, catalase and glutathione and an increase in TBARS levels as compared to group 5 rats.

Administration of anti-TB drugs for 45 days to group 2 showed cell swelling, cytoplasmic vacuolation and occasional areas of infiltration of inflammatory cells on histological examination of rat liver. Concurrent administration of Vedic Guard to group 3 showed normal photomicroscopic appearance of hepatic parenchymal tissue including portal areas. The microscopic examination of rat liver treated with anti-TB drugs for 45 days with a recovery period of 20 days showed minor pathological changes like cell swelling reduced sinusoidal spaces and vacuolation of occasional hepatocytes. Treatment with Vedic Guard for 20 days showed normal appearance of liver parenchyma including portal areas.

DISCUSSION

Co-administration of Vedic Guard along with anti-TB

drugs significantly prevented all the biochemical and histological alterations caused by anti-TB drugs. A recovery period of 20 days after anti-TB drugs treatment for 45 days failed to abolish the degenerative changes in liver, whereas treatment with Vedic Guard for 20 days reversed the changes in the levels of all the biochemical parameters and histological changes.

Vedic Guard, a polyherbal formulation is a synergistic combination of 16 medicinal plant extracts including *Eclipta alba, Emblica officinalis, Tinospora cordifolia, Glycrrhiza glabra, Terminalia arjuna, Curcuma longa* etc. These are well known in the Ayurveda system of medicine. Alcoholic extracts of Ebmlica officinalis have been reported to posses hepatoprotective effects through a membrane stabilizing, an antioxidant and a CYP2E1 inhibitory mechanism (17). Wedelolactone and coumestan derivatives isolated from *Eclipta alba* have reported to possess an antihepatotoxic effect (18) probably by regulating the levels of hepatic microsomal drug metabolizing enzymes (19). An alpha-D-glucagon exhibiting unique immune stimulating property was isolated from *Tinospora cordifolia* (20). Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl_4 intoxicated rats too have been reported (21). *Glycyrrhiza glabra* contains a chalcone derivative and seven phenolic compounds which are very potent antioxidant agents (22) and act as chemoprotective agents. Hypercholesterolaemic and antioxidant effects of *Glycyrrhiza glabra* have been demonstrated to be mediated via accelerated cholesterol, neutral sterols and bile acid elimination through fecal matter and increased activity of hepatic SOD, catalase and increased ascorbic acid content (23). Aqueous extract of *Terminalia arjuna* have been shown to be rich in tannins and triterpenes which corrects the oxidative stress (24). *Curcuma longa* Linn. has been found to possess a myriad of therapeutic activities ranging from anti-inflammatory, anti-oxidant, anti-hepatotoxic, anti-microbial, chemo protective, antifertility, neuroprotective and HIV-1 and HIV-2 protease inhibitory activity (25)

CONCLUSION

The observations above suggest that the Vedic guard, a polyherbal formulation has a hepatoprotective activity on anti-tubercular drugs induced liver injury in the rats. The mechanism of hepatoprotection may be attributed to its antioxidant activity; this in turn is related to the presence of polyphenols and flavonoids in the formulation.

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