Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Hepatoprotective potential of ethanolic extract of *Ziziphus oenoplia* (L.) Mill roots against antitubercular drugs induced hepatotoxicity in experimental models

Ch V Rao[1*], AKS Rawat[1], Anil P Singh[1], Arpita Singh[2], Neeraj Verma[2]

[1]*Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (Council of Scientific and Industrial Research), Rana Pratap Marg, P.O. Box No. 436, Lucknow 226001, Uttar Pradesh, India*
[2]*Department of Pharmacology, Goel Institute of Pharmacy & Sciences, Faizabad Road (Near Indira Canal) Lucknow 227105, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form 25 September 2011
Accepted 15 October 2011
Available online 20 April 2012

Keywords:
Ziziphus oenoplia (Rhamnaceae)
Rifampicin
Isoniazid
Hepatotoxicity

ABSTRACT

Objective: To evaluate the hepatoprotective potential of ethanolic (50%) extract of *Ziziphus oenoplia* (L.) Mill (*Z. oenoplia*) root against isoniazid (INH) and rifampicin (RIF) induced liver damage in animal models. **Methods:** Five groups of six rats each were selected for the study. Ethanolic extract at a dose of 150 and 300 mg/kg as well as silymarin (100 mg/kg) were administered orally once daily for 21 d in INH + RIF treated groups. The serum levels of glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (SALP), and bilirubin were estimated along with activities of superoxide dismutase, catalase, glutathione S-transferase, glutathione peroxidase, and hepatic melondialdehyde formation. Histopathological analysis was carried out to assess injury to the liver. **Result:** The considerably elevated serum enzymatic activities of glutamic oxaloacetic transaminase, glutamate pyruvate transaminase, alkaline phosphatase and bilirubin due to INH + RIF treatment were restored towards normal in a dose dependent manner after the treatment with ethanolic extract of *Z. oenoplia* roots. Meanwhile, the decreased activities of superoxide dismutase, catalase, glutathione S-transferase and glutathione peroxidase were also restored towards normal dose dependently. In addition, ethanolic extract also significantly prevented the elevation of hepatic melondialdehyde formation in the liver of INH + RIF intoxicated rats in a dose dependent manner. The biochemical observations were supplemented with histopathological examination of rat liver sections. **Conclusions:** The results of this study strongly indicate that ethanolic extract of *Z. oenoplia* has a potent hepatoprotective action against INH + RIF induced hepatic damage in rats.

1. Introduction

The liver, a key organ of metabolism and excretion, is constantly endowed with the task of detoxification. Hepatotoxicants, including viruses, fungal products, bacterial metabolites, minerals, environmental pollutants and chemotherapeutic agents, can induce various disorders of the organ[1]. Many traditional remedies employ herbal drugs for the treatment of liver ailments[2]. In India, a number of medicinal plants and their formulations, such as *Aegle marmelos (A. marmelos), Solanum nigrum (S. nigrum)* and *Ficus carica (F. carica)* are used to cure hepatic disorders in traditional medicine[3-5]. *Ziziphus oenoplia* (L.) Mill. (*Z. oenoplia*) (Family-Rhamnaceae) is one of the folk herbal plant, commonly used in central zone, Uttar Pradesh (India) for hepaoprotective activity[6]. *Z. oenoplia* is a thorny sprawling bush, commonly known as makai in Hindi and jackal jujube in English, widely found in India, Pakistan, Sri Lanka, Malaysia and Australia. Antiulcer activity of *Z. oenoplia* roots in rats has been studied by Suryakant *et al*[7] Antiplasmodial activity of *Z. oenoplia* var. brunoniana was studied and new antiplasmodial

*Corresponding author: Ch V Rao, Pharmacology Laboratory, Pharmacognosy and Ethnopharmacology Division, NBRI, Lucknow 226001, Uttar Pradesh, India.
Tel: + 91- 522 2297976
Fax: +91-522 220583
E-mail: chvrao72@yahoo.com

cyclopeptide alkaloids were found *viz* Ziziphine N, O, P by Sunit *et al*[8]. Chemical investigation of this plant has shown the presence of cyclopeptide alkaloids such as Ziziphine A, B, C, D, E, Abyssinine B and A in stem bark of the plant. A decoction of the root bark is used to promote the healing of fresh wounds. The fruit is used as an ingredient in the preparation of stomach ache pills among the Munda tribe[9]. Isoniazid and rifampicin (INH and RIF), being the first line drugs used as antituberculous chemotherapy, are known to be associated with hepatotoxicity[10]. Oxidative stress produced by INH and RIF causes hepatic injury[11]. The frequency of hepatotoxicity is increased when these drugs are used in combination. Therefore, the present study has been designed to evaluate the hepatoprotective activity of ethanolic extract of *Z. oenoplia* roots against isoniazid and rifampicin induced liver damage in albino rats.

2. Materials and methods

2.1. Plant material

Roots of *Z. oenoplia* were collected from District Hardoi, Uttar Pradesh, (India) in the month of July 2010 and were authenticated by Dr. A. K. S. Rawat Scientist & Head Pharmacognosy and Ethnopharmacology division at National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh (India). A voucher specimen (NBRI/CIF/175/2010) has been deposited in the institute for further reference.

2.2. Animals

Wistar albino rats of either sex weighing 150-200 g were kept at departmental animal house of National Botanical Research Institute, Lucknow, Uttar Pradesh (India) at a temperature (25 ± 2) ℃ and 12 h light/dark cycle respectively for one week before and during the experiments and fed with standard diet and water *ad libitum*. Animal studies were conducted according to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.3. Preparation of extract

Roots of *Z. oenoplia* were washed with distilled water to remove dirt and soil and shade dried in a ventilated place at room temperature. The dried root samples were cut into small pieces and reduced to coarse powder by mechanical grinder, extracted with 50% alcohol as solvent in soxhlet extractor for 18 h. The extract was filtered and concentrated under reduced pressure using rotavapor. The extract was freeze-dried and stored in deep freezer for further use.

2.4. Chemicals and drugs

Isoniazid and rifampicin were purchased from Sigma-Aldrich Company (USA). Silymarin was obtained from Ranbaxy Laboratories Limited, India. All other chemicals were of analytical grade. Serum GOT, GPT and ALP were determined by kinetic method using the kit of Agappae Diagnostic Ltd., India in a double beam spectrophotometer.

2.5. Preliminary phytochemical screening

The ethanolic extract obtained was tested for the presence of various chemical constituents such as saponins, flavonoids, glycosides, alkaloids, tannins and reducing sugar by Trease and Evans[12].

2.6. Isoniazid and rifampicin induced hepatotoxicity

Isoniazid and rifampicin (50 mg/kg body wt. each, p.o) suspension were prepared separately in carboxy methyl cellulose (CMC). Rats were treated with isoniazid (INH), co-administered with rifampicin (RIF) for 21 d orally to produce hepatotoxicity[13].

2.7. Experiment design

Male wistar albino rats were divided into five groups comprising six animals in each group.

Group I (NC): Normal control

Group II (HC): Hepatotoxic control received INH+RIF

Group III (HCE1): Animals were given INH+RIF+*Z. oenoplia* (150 mg/kg)

Group IV (HCE2): Animals were given INH+RIF+*Z. oenoplia* (300 mg/kg)

Group V (HCSD): Animals were given INH+RIF+Silymarin (100 mg/kg)

All the treatments were given orally in CMC (1%) in distilled water (10 mL/kg) by means of orogastric cannula for 21 d. At the end of the treatment, blood samples of all animals were collected in sterile centrifuge tubes and allowed to clot. Serum was separated and used for the assay of serum marker enzymes.

2.8. Biochemical determinations

The biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and serum bilirubin (SB) were assayed according to standard methods using an assay kit[14-16]. The activities

of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione peroxidase (GPx) were determined by the method of Khatun *et al*, Ebadi *et al*, Dhanasekaran *et al* and Sindhu *et al* respectively[17-20]. The contents of malondialdehyde (MDA) were determined by the method of Chaurasia[21].

2.9. Histopathology

Liver samples from all animals were processed for light microscopy. Tissue sections were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin (H&E) and Masson's trichrome stain. The degree of liver damage was examined blindly by a liver pathologist under a light microscope. The sections were examined for the pathological findings of hepatotoxicity such as centrilobular necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, *etc*.

2.10. Statistical analysis

The data are expressed as mean ± S.E.M. of six observations. The results were obtained statistically analyzed by Student's *t*-test. Value $P<0.05$ was taken as the criterion of significance.

3. Results

3.1. Phytochemical screening

The ethanolic extract was tested and showed presence of alkaloids, carbohydrates, saponins, flavanoids, anthocyanins and tannins.

3.2. Effect of ethanolic extract on biochemical parameters

The results of hepatoprotective effects of ethanolic extract of *Z. oenoplia* on INH + RIF intoxicated rats are shown in Table 1. Administration of INH + RIF at a dose of 50 mg/kg bw p.o. each significantly ($P<0.05$) elevated SGPT, SGOT, SALP, SB activities when compared to the normal control. Treatment of ethanolic extract of *Z. oenoplia* at a dose of 150 mg/kg and 300 mg/kg bw, 1 h prior to INH + RIF administration significantly ($P<0.01$ to $P<0.001$) protected the elevation of transaminases and SALP activities towards normal. Serum bilirubin (SB) was significantly ($P<0.01$) reduced by administration of *Z. oenoplia* at a dose of 150 mg/kg and 300 mg/kg as compared to hepatotoxic controlled rats.

3.3. Effect of ethanolic extract on antioxidant parameters

Activities of hepatic SOD, CAT and GPx were given in the Table 2. SOD and GPx activities were significantly ($P<0.01$) enhanced after the treatment of *Z. oenoplia* + INH + RIF treated group. However, the hepatic CAT activity was improved significantly ($P<0.01$) when compared to the hepatotoxic control. Further the activity of GST was enhanced and normalized in the *Z. oenoplia* + INH + RIF treated. Hepatic MDA level was significantly ($P<0.05$) elevated in INH + RIF control group than the normal group. It was significantly ($P<0.01$ to $P<0.001$) reduced by administration of *Z. oenoplia* at a dose of 150 mg/kg and

Table 1
Effect of ethanolic extract of *Z. oenoplia* in different biochemical parameters in INH + RIF induced-hepatotoxic rats.

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	SB (mg/dL)
NC	45.02 ± 5.25	75.56 ± 3.12	139.2 ± 2.1	0.53 ± 0.03
HC	145.26 ± 6.12[a]	131.34 ± 6.39[a]	275.6 ± 1.82[a]	1.22 ± 0.81[a]
HC+E1	102.11 ± 2.44[b]	104.12 ± 4.42[b]	179.2 ± 2.90[b]	1.10 ± 0.12[b]
HC+E2	69.27 ± 1.43[c]	85.12 ± 2.56[c]	116.1 ± 3.80[c]	0.89 ± 1.20[c]
HC+SD	65.41 ± 4.71[b]	82.42 ± 7.31[b]	102.7 ± 3.98[b]	0.85 ± 0.59[b]

Data are expressed as mean ± S.E.M ($n = 6$). [a]$P < 0.01$ as compared with normal rats. [b]$P < 0.001$ and [c]$P < 0.0001$ as compared with INH+ RIF treated group.

Table 2
Effect of ethanolic extract of *Z. oenoplia* in different antioxidants parameters in INH + RIF induced-hepatotoxic rats.

Groups	GPx (U/mg)	CAT (U/mg)	SOD (U/mg)	GST nmol/min/mg protein	MDA μ mol/mg protein
NC	29.55 ± 1.34	20.72 ± 3.12	22.39 ± 1.30	271.2 ± 12.9	22.5 ± 5.1
HC	24.20 ± 1.55[a]	12.72 ± 1.86[a]	16.04 ± 3.62[a]	200.5 ± 11.2[a]	79.3 ± 8.7[a]
HC+E1	23.75 ± 1.94[b]	14.02 ± 1.53[b]	19.84 ± 1.11[b]	197.5 ± 12.9[b]	49.5 ± 4.6[b]
HC+E2	29.18 ± 1.21[c]	16.98 ± 0.86[c]	20.09 ± 2.04[c]	207.8 ± 10.3[c]	32.7 ± 7.1[c]
HC+SD	27.62 ± 2.01[b]	18.44 ± 1.51[b]	21.78 ± 1.27[b]	228.2 ± 13.2[b]	28.7 ± 4.9[b]

Data are expressed as mean ± S.E.M ($n = 6$). [a]$P < 0.01$ as compared with normal rats. [b]$P < 0.001$ and [c]$P < 0.0001$ as compared with INH+ RIF treated group.

300 mg/kg as compared to hepatotoxic rats.

3.4. Effect of ethanolic extract on histopathology of liver

Histopathological analysis of the INH + RIF treated animal showed severe centrilobular necrosis, fatty infiltration and lymphocytes infiltration. NC Group: Hepatocytes of the normal control group showed a normal lobular architecture of the liver (Figure 1). HC Group: In the INH + RIF treated group, liver showed hepatocytic necrosis and inflammation also observed in the centrilobular region with portal triaditis (Figure 2). HCE1 group: Ethanolic extract of *Z. oenoplia* treated group at a dose of 150 mg/kg showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal (Figure 3). HCE2 group: Ethanolic extract of *Z. oenoplia* treated group at a dose of 300 mg/kg showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal (Figure 4). HCSD group: Silymarin treated group at a dose of 100 mg/kg showed normal hepatocytes and their lobular architecture was normal (Figure 5).

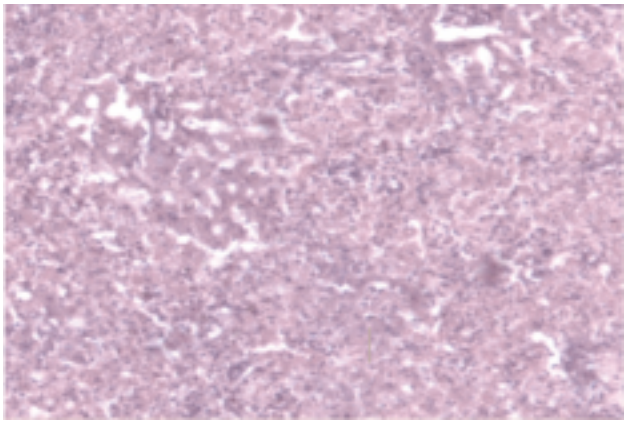

Figure 1. Normal rats (Group I) showed normal hepatocytes with well preserved cytoplasm with normal lobular structural design of the liver.

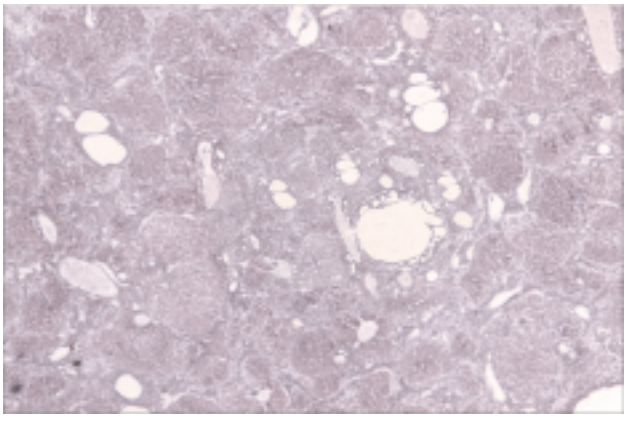

Figure 2. Hepatocytes of INH+RIF (50 mg/kg, each p.o.) treated rats (Group II) showing massive fatty changes, necrosis ballooning degeneration, and broad infiltration of the lymphocytes and kupffer cells around the central vein and the loss of cellular boundaries.

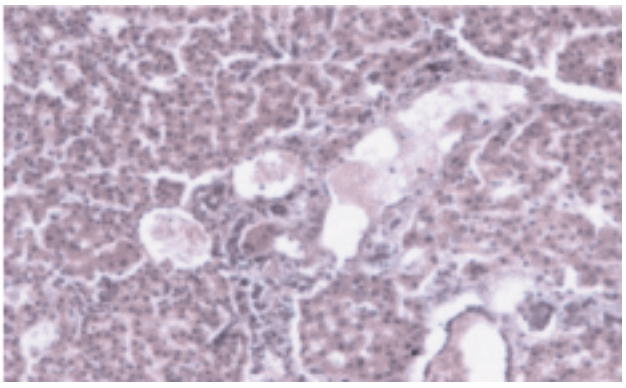

Figure 3. Hepatocytes of rats treated with INH+RIF (50 mg/kg, each p.o.) + ethanolic extract of *Z. oenoplia* (150 mg/kg, p.o.)×21 d (Group III) showing well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus.

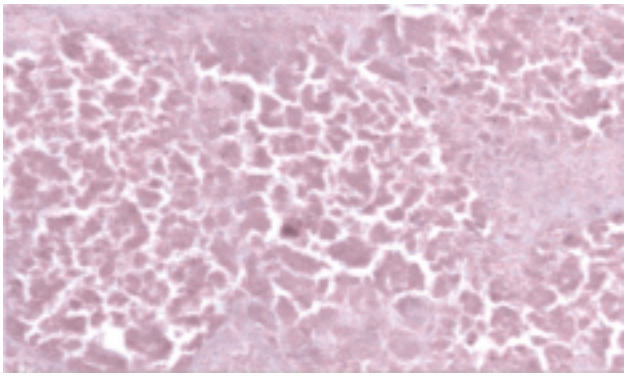

Figure 4. Liver section of rats treated with INH+RIF (50 mg/kg, each p.o.) + ethanolic extract of *Z. oenoplia* (300 mg/kg, p.o.)×21 d (Group IV) showing well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus.

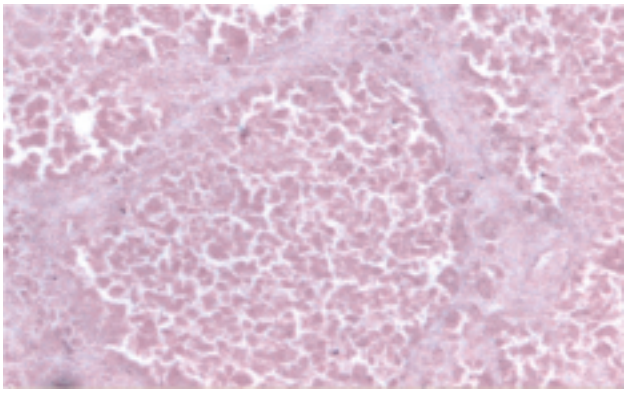

Figure 5. Liver section of rats treated with INH+RIF (50 mg/kg, each p.o.) + Silymarin (100 mg/kg, p.o.)×21 d (Group V) showing well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus.

4. Discussion

The liver is a vital organ and injured by number of chemicals and drugs. In the present study, hepatotoxicity model in wistar rats was successfully produced by

administering INH and RIF (50 mg/kg per day each) orally. Increase in the normal upper limits in the measured serum transaminases of INH+RIF group on day 21 of the experiment was a biochemical indication of liver injury. During the metabolism of INH, hydrazine is produced directly (from INH) or indirectly (from acetyl hydrazine). Hydrazine play a pivotal role in INH-induced liver damage in rats, which is consistent with the report by Sarich *et al*[22]. INH is metabolized in liver primarily by acetylation and hydrolysis, and these acetylated metabolites are thought to be hepatotoxins[23]. The combination of INH and RIF was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation and cytochrome P450 was thought to be involved the synergistic effect of RIF on INH[24].

Metabolism of chemicals takes place largely in the liver, which accounts for the organ's susceptibility to metabolism-dependent, drug induced injury. The drug metabolites can be electrophilic chemicals or free radicals that undergo or promote a variety of chemical reactions, such as the depletion of reduced glutathione; covalently binding to proteins, lipids, or nucleic acids; or inducing lipid peroxidation[25]. Earlier report also says that there did not seem to be clear evidence that isoniazid proves much more injuries than rifampicin and in this connection, they consider that it is the combination of these two drugs that confer the additive, or even synergistic, potential of liver toxicity than either agent alone, as conjectured[26-28]. Hydrazine metabolite of INH and is subsequent effect on CYP2E1 induction is involved in the development of INH-induced hepatotoxicity in rats[29] and also oxidative stress as one of the mechanism for INH + RIF induced hepatic injury[30]. Administration of ethanolic extract of *Z. oenoplia* at a dose of 150 mg/kg and 300 mg/kg improved liver function by decreasing the serum GOT, GPT and alkaline phosphate levels in hepatotoxic rats significantly, which is almost comparable to group treated with silymarin, a potent hepatoprotective drug used as reference standard. Total bilirubin, a byproduct of the breakdown of red blood cells in the liver, bilirubin is a good indicator of liver function. High levels will cause icterus (jaundice) and are indicative of damage to the liver and bile duct[31]. Treatment with ethanolic extract of *Z. oenoplia* reduced the serum ALP as well as the total bilirubin levels in INH + RIF induced hepatic injury, indicating its protective effect over liver and improvement in its functional efficiency. Hepatocellular disintegrate and the inflammation in the liver was observed in the centrilobular region by histopathological examination in INH + RIF treated groups[32-41]. The above observations strongly indicate the hepatoprotective activity of ethanolic extract of *Z. oenoplia* against INH+RIF intoxicated rats. The hepatoprotective effect of the plant extract might be attributed to the presence of unique chemical classes such as alkaloids[42] and polyphenols[43]. Therefore it is concluded that effects of ethanolic extract of *Z. oenoplia* on liver protection is releated to glutathione mediated detoxification as well as free radical scavenging activity. Thus, this study represents a novel and attractive idea to prevent INH+RIF induced hepatic injury.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

Authors are thankful to Department of Science & Technology (DST), New Delhi for partial financial support and Director National Botanical Research Institute (NBRI), Lucknow for providing necessary facilities.

References

[1] Tao W, Ning LS, Wei DZ, Hui LL, Guo CL, Bo JY, et al. Protective effects of dehydrocavidine on carbon tetrachloride-induced acute hepatotoxicity in rats. *J Ethnopharmacol* 2008; **117**: 300-308.

[2] Etuk EU, Agaie BM, Ladan MJ, Garba I. The modulatory effect of *Cochlospermum tinctorium* a rich aqueous root extract on liver damage induced by carbon tetrachloride in rats. *Afr J Pharm Pharmacol* 2009; **3**(4): 151-157.

[3] Singanan V, Singanan M, Begum H. The hepatoprotective effect of Bael leaves (*Aegle marmelos*) in alcohol induced liver injury in albino rats. *Int J Sci Tech* 2007; **2**(2): 83-92.

[4] Hsieh CS, Ko CY, Chen SY, Liu TM, Wu JS, Hu CH, et al. *In-vivo* long-term continuous observation of gene expression in zebrafish embryo nerve systems by using harmonic generation microscopy and morphant technology. *J Biomed Opt* 2008; **13**(6): 064041

[5] Krishna MG, Pallavi E, Ravi KB, Ramesh M, Venkatesh S. Hepatoprotective activity of *Ficus carica* (Linn) leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. *DARU* 2007; **15**(3): 162-167.

[6] Pundir R, Singh G, Pandey AA, Saraf SA. Demand of herbal hepatoprotective formulations in Lucknow-A Survey. *The Pharm Res* 2009; **1**: 23-33.

[7] Jadhav SA, Prassanna SM. Evaluation of antiulcer activity of *Ziziphus oenoplia* (L) Mill. roots in rats. *Asian J Pharm Clin Res* 2011; **1**(1): 92-95.

[8] Suksamrarn S, Suwannapoch N, Aunchai N, Kuno M, Ratananukul P, Haritakun R, et al. Ziziphine N, O, P and Q, new antiplasmodial cyclopeptide alkaloids from *Ziziphus oenoplia* var.brunoniana. *Tetrahedron* 2005; **61**: 1175-1180.

[9] Singh MP, Panda H. *Medicinal herbs with their formulations*, vol 1. Delhi: Daya Publishing House; 2005, p. 97-100.

[10] Tasduq SA, Peerzada K, Koul S, Bhat R, Johri RK. Biochemical manifestations of antituberculosis drugs induced hepatotoxicity and the effect of silymarin. *Hepatol Res* 2005; **31**: 132-135.

[11]Jehangir A, Nagi AH, Shahzad M, Azam Z. The hepato-protective effect of *Cassia fistula* (amaltas) leaves in isoniazid and rifampicin induced hepatotoxicity in rodents. *Biomedica* 2010; **26**: 25-29.

[12]Trease GE, Evans WC. *Trease and evans pharmacognosy*. 15th Ed. Edinburgh: Saunders; 2002, p. 249, 454.

[13]Pal R, Vaiphei K, Sikander A. Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. *World J Gastroenterol* 2006; **12**(4): 636-639.

[14]Daryoush M, Bahram AT, Yousef D, Mehrdad N. Protective effect of turnip root (*Brassica Rapa*. L) ethanolic extract on early hepatic injury in alloxanized diabetic rats. *Aust J Basic Appl Sci* 2011; **5**(7): 748-756.

[15]Karthikeyan M, Wawdhane SS, Kannan M, Rajasekar S. Hepatoprotective activity of ethanolic extract of *Spermacoce Hispida*. Linn against carbon tetrachloride (CCl_4) induced hepatotoxicity on albino wistar rats. *Int J Pharm Res Dev* 2011; **2**(11): 45-52.

[16]Nassar MI, Gaara AH, Ghorab AHEl, Farrag ARH, Shen H, Huq E, et al. Chemical constituents of clove (*Syzygium aromaticum*, Fam. Myrtaceae) and their antioxidant activity. *Rev Latinoamer Quím* 2007; **35**(3): 47-57.

[17]Khatun S, Chatterjee NC, Cakilcioglu U. Antioxidant activity of the medicinal plant *Coleus forskohlii Briq*. *Afr J Biotechnol* 2011; **10**(13): 2530-2535.

[18]Shokrzadeh M, Ebadi AG, Mirshafiee SS, Choudhary MI. Effect of the aqueous green leaf extract of green tea (*Camellia sinensis*) on glucose level of rat. *Pak J Biol Sci* 2006; **9**(14): 2708-2711.

[19]Dhanasekaran JJ, Ganapathy M. Hepatoprotective effect of *Cassia auriculata* L. leaf extract on carbon tetrachloride intoxicated liver damage in Wistar albino rats. *Asian J Biochem* 2011; **6**(1): 104-112.

[20]Sindhu T, Rajamanikandan S, Ragavendran P, Sophia D, Meenaksh P, Priya DD, et al. Antidiabetic activity of *Mollugo nudicaulis* against alloxan induced diabetic rats. *Int J Appl Biol Pharm Tech* 2010; **1**(3): 511-519.

[21]Chaurasia S. Anti-inflammatory and antioxidant activity of *Strychnos nux vomica* Linn. *Am Eurasian J Sustain Agric* 2009; **3**(2): 244-252.

[22]Garner P, Holmes A, Ziganahina L. Tuberculosis. *Clin Evid* 2004; **11**: 1081-1093.

[23]Ramaiah SK, Apte U, Mehendale HIM. Cytochrome P4502E1 induction increases thioacetamide liver injury in diet-restricted rats. *Drug Metab Dispos* 2001; **29**: 1088-1095.

[24]Shakun NP, Shman'ko VV. Synergistic effect of rifampicin on hepatotoxicity of isoniazid. *Antibiot Med Biotekhnol* 1985; **30**: 185-189.

[25]Girling DJ. Adverse effects of antituberculosis drugs. *Drugs* 1982; **23**(1-2): 56-74.

[26]Yasuda K, Sato A, Chida K. Pulmonary tuberculosis with chemotherapy related liver dysfunction. *Kekkadu* 1990; **65**: 407-413.

[27]Wu JW, Leev SD, Yeh PF. Isoniazid-rifampicin induced hepatitis in hepatitis B carriers. *Gastroentrology* 1990; **98**: 502-504.

[28]Steele MA, Burk RF, Des Prez RM. Toxic hepatitis with isoniazid and rifampicin: a meta-analysis. *Chest* 1991; **99**: 465-471.

[29]Jiang Y, Ren-xiu P, Jing Y, Rui K, Juan L. CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. *Acta Pharmacol Sin* 2004; **25**(5): 699-704.

[30]Peretti E, Karlaganis G, Lauterburg BH. Acetylating of acetylhydrazine, the toxic metabolite of isoniazid, in humans: inhibition by concomitant administration of isoniazid. *J Pharmacol Exp Ther* 1987; **243**: 686-689.

[31] Rajesh KG, Achyut NK, Geeta W, Murthy PS, Ramesh C, Vibha T. Nutritional and hypoglycemic effect of fruit pulp of *Annona squamosa* in normal healthy and alloxan-induced diabetic rabbits. *Ann Nutr Metab* 2005; **49**: 407-413.

[32]Ravinder P, Kim V, Arbab S, Kartar S, Satya VR. Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. *World J Gastroenterol* 2006; **12**(4): 636-639.

[33]Kaplowitz N. Drug-induced liver disorders: introduction and overview. In: Kaplowitz N, DeLeve LD, editors. *Drug-induced liver disease*. New York: Marcel Dekker; 2002, p. 1-13.

[34]Madhu Kiran P, Vijaya Raju A, Ganga Rao B. Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol-induced hepatotoxicity in rat. *Asian Pac J Trop Biomed* 2012; **2**: 352-356.

[35]Singh K, Singh N, Chandy A, Manigauha A. *In vivo* antioxidant and hepatoprotective activity of methanolic extracts of *Daucus carota* seeds in experimental animals. *Asian Pac J Trop Biomed* 2012; **2**: 385-388.

[36]Jain M, Kapadia R, Jadeja RN, Thounaojam MC, Devkar RV, Mishra SH. Cytotoxicity evaluation and hepatoprotective potential of bioassay guided fractions from *Feronia limmonia* Linn leaf. *Asian Pac J Trop Biomed* 2011; **1**: 443-447.

[37]Ravikumar S, Gnanadesigan M. Hepatoprotective and antioxidant activity of a mangrove plant *Lumnitzera racemosa*. *Asian Pac J Trop Biomed* 2011; **1**: 348-352.

[38]Sengupta M, Dutta Sharma G, Chakraborty B. Hepatoprotective and immunomodulatory properties of aqueous extract of *Curcuma longa* in carbon tetra chloride intoxicated Swiss albino mice. *Asian Pac J Trop Biomed* 2011; **1**: 193-199.

[39]Devaraj VC, Gopala Krishna B, Viswanatha GL, Kamath JV, Kumar S. Hepatoprotective activity of Hepax-A polyherbal formulation. *Asian Pac J Trop Biomed* 2011; **1**: 142-146.

[40]Hussain T, Gupta RK, Sweety K, Khan MS, Md S Hussain, Md Arif, et al. Evaluation of antihepatotoxic potential of *Solanum xanthocarpum* fruit extract against antitubercular drugs induced hepatopathy in experimental rodents. *Asian Pac J Trop Biomed* 2011; **2**: 454-460.

[41]Thirumalai T, David E, Viviyan Therasa S, Elumalai EK. Restorative effect of *Eclipta alba* in CCl_4 induced hepatotoxicity in male albino rats. *Asian Pac J Trop Dis* 2011; **1**: 304-307.

[42]Pradeep HA, Khan S, Ravikumar K, Ahmed MF, Rao MS, Kiranmai M, et al. Hepatoprotective evaluation of *Anogeissus latifolia: In-vitro* and *in-vivo* studies, *World J Gastroenterol* 2009; **15**(38): 4816-4822.

[43]Masella R, Benedetto RD, Vari R, Filesi C, Giovannini C. Novel mechanism of natural antioxidants compounds in biological system: involvement of glutathione related enzymes. *J Nutr Biochem* 2005; **16**: 577-586.