

Received: 17th Feb-2013

Revised: 25th Feb-2013

Accepted: 26th Feb-2013

Research article

EFFECT OF ETHANOLIC EXTRACT OF *PHYLLANTHUS AMARUS* AND *TYLOPHORA INDICA* ON ISONIAZID INDUCED HEPATIC INJURY IN WISTAR ALBINO RATS.

Shyamjith M¹ and Rao SN^{1*}

Dept of Pharmacology, Yenepoya Medical College, Yenepoya University, Mangalore-18, Karnataka.

E-mail id: silentkillersm@gmail.com

ABSTRACT

Objective: To assess the hepatoprotective effect of ethanolic extract of leaves and stem of *Phyllanthus amarus* and ethanolic extract of leaves of *Tylophora indica* against Isoniazid induced liver toxicity in experimental animals. **Methods:** Liver toxicity was induced by administering Isoniazid 27mg/kg orally for 30 days in Wistar albino rats. Ethanolic (90%) extracts of *Phyllanthus amarus* (PAEE) and *Tylophora indica* (TIEE) was administered orally to the experimental animals for 30days. The hepatoprotective activity of the extracts was assessed by analyzing the levels of various biochemical parameters like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -Glutamyl transferase (GGT), total bilirubin (TBL) and albumin (ALB) in serum. Mean while the levels of antioxidant enzymes like Superoxide dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH) were measured in rat liver homogenate. **Results:** The results showed that on administration of Isoniazid for 30 days caused a significant increase ($p < 0.001$) in the levels of ALT, AST, ALP, GGT, TBL in serum. At the same time, the serum level of ALB was significantly ($p < 0.01$) reduced in Isoniazid administered rats. The levels of SOD, CAT and GSH in liver homogenate were also decreased significantly ($p < 0.01$) in Isoniazid administered animals. The levels of above biochemical parameters were significantly ($p < 0.001$) reversed in rats which received PAEE and TIEE. **Conclusion:** The present study proves that the ethanolic extracts of *Phyllanthus amarus* and *Tylophora indica* have a significant protective action against isoniazid induced hepatic injury.

Keywords: Isoniazid, Liver injury, Wistar albino rats, *Phyllanthus amarus*, *Tylophora indica*, ethanolic extract, Hepatoprotection.

INTRODUCTION

Tuberculosis [TB] has become an enormous problem worldwide. The most effective method to cure this deadly infection is the treatment with antituberculosis drugs (WHO Report, 1993). The mortality rates are high in TB patients without treatment. Treatment using the combinations of anti-TB drugs was developed by around 1940s and has dramatically reduced mortality rates among TB patients (WHO report, 2011). By late 1990s, the Government of India has implemented the Revised National TB Control Programme [RNTCP], the globally recommended Directly Observed treatment Strategy [DOTS] for the treatment of TB patients (Pauline Joseph et al, 2011). Isoniazid, the first line anti-TB drug is an essential component of DOTS regimen (ICMR Bulletin, 2001). The major hurdle in the treatment of TB is the poor patient compliance due to adverse effects of the anti-TB drugs. The important first line anti-TB drug Isoniazid is not free from the incidence of adverse drug reactions. Isoniazid induced liver injury is one of the pit falls in DOTS, necessitating discontinuation of the therapy (Earl Hershfield, 1999; Alma Tostmann, et al, 2008). Drug-induced liver injury [DILI] is a matter of concern that affects a large population of patients and physicians. It also has an impact on the pharmaceutical industry, and government regulatory bodies (Naga Chalasani et al, 2008). In spite of tremendous advancement in allopathic medicine, there is no effective liver protective agent in drug armory. Here comes the importance of natural products. Natural products play an important role in the field of new drugs research and development (Kong JM et al, 2003). The use of natural remedies for the treatment of liver ailments has a long history, starting with the traditional Ayurvedic treatment, to the Chinese and other systems of traditional medicines. A large number of indigenous plants have hepatoprotective activity (Mohamed Saleem TS et al, 2010). In traditional Indian medicinal system several plants belonging to the family of Euphorbiaceae and Asclepiadaceae are used for the treatment of liver ailments.

Phyllanthus amarus (Syn.*Phyllanthus niruri*) is a perennial herb distributed throughout the tropical and subtropical regions of both hemispheres. It belongs to *Euphorbiaceae* family. *Phyllanthus amarus* has a long history in herbal medicine systems all over the world. This important medicinal herb is used for curing a wide range of ailments like jaundice, asthma, hepatitis, urogenital problems, dysentery, dyspepsia, arthritis malaria, etc (Rashmi Mathur,2011; Rajeshwar Y et al, 2008). It has also shown activity against Hepatitis B and C virus (Venkateswaran PS et al, 1987; Bhattacharyya et al, 2003)

Tylophora indica is a slender climbing perennial plant. This plant of *Asclepiadaceae* family is seen in southern and eastern part of India in plains, forests, and hilly places. Plant is traditionally used in the treatment of bronchial asthma, bronchitis, rheumatism, allergies, inflammation, dysentery, whooping cough, and diarrhea. (Kumar Sunil et al,2012; Sabitha Rani et al,2012; Harmanjit Kaur et al,2012). There are various animal studies reporting the hepatoprotective activity of these plants on various chemicals induced liver injury (Bhattacharjee R et al,2007; Vipul B Gujarati et al,2007; Narayan P. Yadav et al 2008; Mujeeb et al,2009; Malathi R et al,2011). To the best of our knowledge, none of them have reported their role in antitubercular drug induced liver injury. So in this study the role of *Phyllanthus amarus* and *Tylophora indica* in Isoniazid induced liver injury was investigated.

MATERIALS AND METHODS

Drugs and Chemicals

Isoniazid (Macleods,Daman), Silymarin (Micro labs , Bangalore) were obtained from a pharmacy in Mangalore. All the chemicals used for biochemical analysis were of analytical grade and was procured from Himedia, India.

Instruments

Soxhlet apparatus

Autoanalyser

UV Spectrophotometer.

Plant material

The plants were cultivated during the month of June. The fresh leaves and stem from the young *Phyllanthus amarus* and fresh leaves from *Tylophora indica* were collected in the month of September. They were authenticated by Dr.Noeline J.Pinto, Head of Botany department, St.Agnes College, Mangalore, Karnataka, India.

They were shade dried, and then grinded into coarse powder.

Preparation of the extracts

***Phyllanthus amarus* ethanolic extract (PAEE):** A weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90 %) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60°C. The percentage yield of the extract was 20%. The ethanolic extract was dissolved in distilled water.

***Tylophora indica* ethanolic extract (TIEE):**

A weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90 %) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60°C. The percentage yield of the extract was 10%. The ethanol extract was dissolved in distilled water.

Animals

Adult Wistar albino rats of either sex weighing 175-200 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC), Yenepoya University. The rats were maintained under standard conditions in the Animal House (CPCSEA approved, Reg No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The rats were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard housing conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water *ad libitum*. The rats were maintained on a 12:12 hour light-dark cycle.

Acute oral toxicity studies

Acute toxicity study was performed according to OECD guidelines No: 423 (OECD, 2000). Overnight fasted Wistar albino rats of either sex were administered PAEE and TIEE orally as a single dose at different dose levels of 100, 200, 400, 800, 1600 and 3200 mg/kg body weight. Animals were closely monitored for first 2 hours, for the symptoms of toxicity and death. After 2 hours they were given food and water *ad libitum*. There after they were monitored for next 14 days for any kind of toxicity.

Experimental protocol

The rats were divided into five groups with 12 animals in each group. Group I received distilled water orally and served as a normal control. Group II received Isoniazid 27mg/kg body weight (Kale BP et al, 2003) for 30days orally in distilled water. This group served as disease control. Group III received PAEE 70mg/kg body weight (Mary Chatterjee et al, 2006) in distilled water orally along with Isoniazid administration. Group IV received TIEE 300mg/kg body weight (Vipul Gujrathi et al, 2007) in distilled water orally along with Isoniazid administration. Group V received Silymarin, a known hepatoprotective agent, 100mg/kg body (Sethuraman MG, 2003) weight in distilled water orally along with Isoniazid administration. This group served as standard control.

On 31st day blood samples of the animals were taken by cardiac puncture under ketamine anesthesia (150mg/kg bodyweight, I.P). Once the blood was withdrawn, the animals were sacrificed by high dose of ketamine (300mg/kg bodyweight I.P). The liver was dissected out for preparing homogenate.

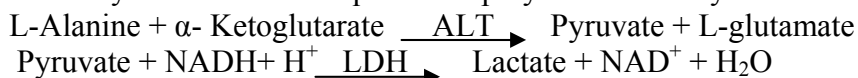
Assessment of hepatoprotective activity

The blood which was drawn under ketamine anesthesia was allowed to clot and the serum was separated at 3000 rpm for 10minutes. The serum was used for the assay of Alanine aminotransferase [ALT], Aspartate aminotransferase [AST], Alkaline phosphatase [ALP], Gamma Glutamyl transpeptidase [GGT], Total Bilirubin [TBL], Albumin [ALB].

Estimation of Alanine aminotransferase levels

ALT level in serum was estimated by UV-Kinetic method. This method is based on the oxidation of NADH by lactate dehydrogenase (LDH).

The enzymatic reaction sequence employed in the assay of ALT is as follows.

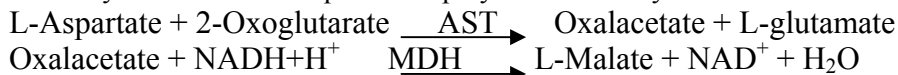


The pyruvate formed in the reaction is reduced to lactate in the presence of Lactate dehydrogenase (LDH) and NADH. The activity of ALT is determined by measuring the rate of oxidation of NADH at 340 nm using spectrophotometer with a temperature controlled cuvette (Bruce R et al, 1958).

Estimation of Aspartate aminotransferase levels

AST level in serum was estimated by UV-Kinetic method by using a coupled reaction of malate dehydrogenase (MDH) and NADH.

The enzymatic reaction sequence employed in the assay of AST is as follows.

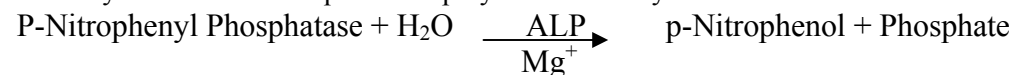


AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate. The Oxaloacetate formed in the first reaction is then reacted with NADH in the presence of malate dehydrogenase (MDH). AST activity is determined by measuring the rate of oxidation of NADH at 340 nm using spectrophotometer with a temperature controlled (Bruce R et al, 1958).

Estimation of Alkaline Phosphatase levels

ALP level in serum was estimated by UV-Kinetic method by measuring the rate of hydrolysis of various phosphate esters.

The enzymatic reaction sequence employed in the assay of ALP is as follows.

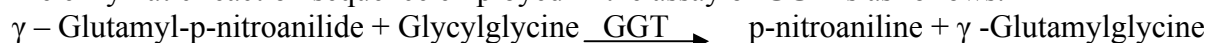


P-Nitrophenyl Phosphatase is colorless but p-Nitrophenol has strong absorbance at 405nm. The rate of increased absorbance at 405 nm is proportional to the enzyme activity. ALP activity is determined by measuring the rate of absorbance at 405 nm using spectrophotometer with a temperature controlled cuvette (Tietz NW, 1983).

Estimation of γ – Glutamyltransferase levels

GGT level in serum was estimated by Kinetic procedure using a synthetic substrate γ – Glutamyl-p-nitroanilide which is activated by Glycylglycine.

The enzymatic reaction sequence employed in the assay of GGT is as follows.



GGT catalyses the transfer of γ – Glutamyl group from γ – Glutamyl-p-nitroanilide. The rate of liberation of p-nitroaniline is directly related to the GGT activity in the sample and is quantitated by measuring the increase in absorbance at 405nm using spectrophotometer with a temperature controlled cuvette (Whitfield JB,2001 ;Rosaiki SB., et al, 1970).

Estimation of Serum Bilirubin

Bilirubin is estimated by reacting it with diazotized sulfanilic acid obtained from sodium nitrate and sulfanilic acid; it forms a pink colored azo compound. Direct bilirubin (conjugated or soluble fraction) reacts very quickly and is read by measuring color developed in 30 seconds of reaction. The conjugated or free bilirubin takes longer time to react and requires an accelerator. Hence total bilirubin is measured using Caffeine as an accelerator allowing the reaction to proceed for 5 minutes and read absorbance of blank and test sample at 546 nm using Spectrophotometer (Patterson J et al,1952) .

Estimation of Albumin

Albumin is an important binding and transport protein for various substances in plasma. Measurement of albumin in serum is used for diagnosis and monitoring of liver diseases. Albumin levels in serum were estimated by BCG method. Serum albumin in the presence of bromocresol green at a slightly acidic pH produce a color change of the indicator from yellow-green to green blue complex. The blue-green colour formed is proportional to the concentration of albumin present, when absorbance is measured spectrophotometrically at 625 nm (Doumas BT et al,1981;Vratislav Chromy' et al,2009).

Assessment of antioxidant activity

The liver which was dissected out was washed immediately with distilled water to remove blood. It was used for preparing homogenate.

Estimation of Superoxide Dismutase level in liver homogenate

10% liver homogenate (1g liver in 10mL of 0.4M phosphate buffer, pH 7.0) was prepared using a glass homogenizer. It was centrifuged at 10,000rpm for 15 minutes. The supernatant was used to estimate the levels of antioxidant enzymes like Super oxide dismutase [SOD]. The estimation of superoxide dismutase enzyme is carried out by Beauchamp and Fridovich method. The substrate used for the assay consists of nitro blue tetrazolium chloride (NBT) which reacts with superoxide anions produced upon illumination of riboflavin in the presence of methionine as an electron donor, to produce formazan which is a blue colored complex. The SOD present in the sample will act on the superoxide anions produced by riboflavin and thereby reduce the net superoxide anions in the substrate leading to decreased production of formazan manifested by decreased intensity of the blue color formed. The decrease in the formation of formazan read at 560nm using spectrophotometer is directly proportional to the amount of SOD in the sample, 50% decrease in the formation of formosan is taken as one unit of SOD (Beauchamp C and Fridovich I, 1971).

Estimation of Catalase level in liver homogenate

Liver tissue was homogenized in a blender with M/150 phosphate buffer at 1-4°C and centrifuged at 10,000rpm for 15 minutes. Sediment was stirred with cold phosphate buffer; allowed to stand in cold with occasional shaking and then repeating the extraction once or twice. The combined supernatants (sometimes opalescent) were used for the assay. The UV light absorption of hydrogen peroxide solution was measured at 240nm. On decomposition of hydrogen peroxide by Catalase, the absorption decreases with time. The enzyme activity could be arrived at from the decreased time (Goth L ,1991).

Estimation of Glutathione level in liver homogenate

This method is based upon the development of a relatively stable yellow color, when 5, 5'-dithiobis 2-nitro benzoic acid (DTNB) is added to sulphhydryl compounds including glutathione. The reaction mixture contained supernatant of tissue homogenate, a precipitating solution (1.67g glacial m-phosphoric acid, 0.2g of EDTA and 30g of NaCl per 100mL of distilled water), Phosphate solution and DTNB solution. Optical density of the yellowish solution was then measured within 10 minutes at 412nm using a spectrophotometer (Sharma SM et al, 2009).

STATISTICAL ANALYSIS

Statistical Analysis of data for significant variation within the groups was performed using the SPSS statistical software. It was done using one way analysis of variance (ANOVA) and multiple group comparisons were made using Tukey Kramer test using Graph pad Prism software. The values were expressed as mean \pm S.D for 12 samples in each group. *P* value < 0.05 was considered as significant.

RESULTS

Acute toxicity study

There was no mortality among the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 3200 mg/kg. This finding suggests that PAEE and TIEE were safe and non-toxic to rats up to 3200 mg/kg.

Effect on LFT in Isoniazid induced hepatotoxicity

The results (Table:1) showed that on administration of Isoniazid for 30 days caused a significant increase ($p < 0.01$) in the levels of ALT, AST, ALP, GGT, BIL in serum of Isoniazid administered rats (Group II) on comparing with the normal rats (Group I). At the same time, the serum level of ALB was significantly ($p < 0.01$) reduced in Isoniazid administered rats (Group II) on comparing with the normal rats (Group I). The levels of above biochemical parameters were significantly ($p < 0.01$) reversed in rats which received PAEE, TIEE and Silymarin (Group III, IV, and V).

Effect on Antioxidant enzymes in Isoniazid induced hepatotoxicity

The results (Table: 2) showed that on administration of Isoniazid for 30 days caused a significant decrease in the levels of SOD, CAT and GSH in liver homogenate (Group II) on comparing with the normal rats (Group I). The levels of above biochemical parameters were significantly ($p < 0.01$) reversed in rats which received PAEE, TIEE and Silymarin (Group III, IV and V).

Table 1: Effect of PAEE and TIEE on LFT in Isoniazid induced hepatotoxicity

Group	AST (IU/gprot)	ALT (IU/gprot)	ALP (IU/gprot)	GGT (IU/gprot)	ALBUMIN (g/dL)	BILIRUBIN (mg/dL)
I (NS)	41.375 \pm 12.681	51.04 \pm 8.668	225.775 \pm 8.668	2.049 \pm 0.6344	3.397 \pm 0.314	0.405 \pm 0.253
II (H)	242.35 \pm 33.805 ^a	252.275 \pm 38.512 ^a	1202.25 \pm 8.52 ^a	9.262 \pm 3.061 ^a	1.209 \pm 0.133 ^a	2.349 \pm 0.136 ^a
III (PAEE+H)	45.01 \pm 6.685 ^b	67.342 \pm 26.761 ^b	235.01 \pm 5.85 ^b	3.561 \pm 3.2 ^b	2.882 \pm 0.263 ^b	0.6424 \pm 0.2238 ^b
IV (TIEE+ H)	41.107 \pm 10.902 ^b	54.883 \pm 11.299 ^b	234.83 \pm 15.28 b	3.011 \pm 0.062 b	2.994 \pm 0.335 b	0.566 \pm 0.100 b
V (S+H)	55.67 \pm 11.28 ^b	68.22 \pm 4.78 ^b	245.63 \pm 7.74 ^b	3.429 \pm 0.103 ^b	2.821 \pm 0.011 ^b	0.648 \pm 0.015 ^b

N=12, One Way ANOVA followed by Tukey Kramer Multiple Comparisons Test.
Results are expressed as Mean \pm SD.
a \rightarrow p<0.01, considered significant on comparing Group II with Group I.
b \rightarrow p<0.01, considered significant on comparing Group III,IV,V with Group II.

Table 2: Effect of PAEE and TIEE on Antioxidant enzymes in Isoniazid induced hepatotoxicity

Group	SOD (IU/gprot)	CAT (IU/gprot)	GSH (μ g/g Prot.)
I (NS)	537.318 \pm 19.75	17.754 \pm 7.33	1799.98 \pm 81.06
II (H)	33.531 \pm 24.839 ^a	0.725 \pm 0.90 ^a	166.21 \pm 19.6 ^a
III (PAEE+H)	387.277 \pm 74.771 ^b	9.12 \pm 1.38 ^b	1658. \pm 67.41 ^b
IV (TIEE + H)	392.985 \pm 40.1 ^b	9.208 \pm 3.59 ^b	1856.69 \pm 83.1 ^b
V (S+H)	295.67 \pm 13.27 ^b	8.82 \pm 1.97 ^b	1283.63 \pm 57.64 ^b

N=12
One Way ANOVA followed by Tukey Kramer Multiple Comparisons Test.
Results are expressed as Mean \pm SD.
a \rightarrow p<0.01, considered significant on comparing Group II with Group I.
b \rightarrow p<0.01, considered significant on comparing Group III,IV,V with Group II.

DISCUSSION

Tuberculosis in India accounts for more than 20 per cent of the global incident cases (WHO report, 2009). Around 90% of TB cases can be cured by short course regimens using the first line anti-tubercular drugs like Isoniazid, Rifampicin, Pyrazinamide and Ethambutol (WHO report, 2012). The major drawback of six month short course chemotherapy of tuberculosis is the poor patient compliance due to adverse effects of first line drugs. One of the fatal adverse reactions reported during the drug therapy of TB is liver injury. It is a fact that three among the four first line drugs Viz Isoniazid, Rifampicin and Pyrazinamide can produce liver injury (Alma Tostmann, et al, 2008).

In this study the ethanolic extract of *Phyllanthus amarus* and *Tylophora indica* was used to evaluate their hepatoprotective role in Isoniazid induced liver toxicity. Isoniazid induced liver injury is a major problem faced by the physicians during the treatment course of tuberculosis (Mark W Sonderup. 2011). The symptomatic hepatotoxicity following isoniazid administration can be fatal if not intervened in time (Khalili H et al, 2009). Isoniazid induced hepatic damage is considered as an idiosyncratic reactions. These types of reactions will be mediated by IgE or by reactive metabolites. Isoniazid is acetylated by the hepatic enzyme N-acetyltransferase into acetylisoniazid and then hydrolyzed into acetylhydrazine and isonicotinic acid. Acetylhydrazine is further hydrolyzed to hydrazine, or acetylated into diacetylhydrazine. The mechanism of liver injury due to isoniazid is believed to be accumulation of hydrazine the toxic intermediate formed during its metabolism. Hydrazine is in turn converted to nitrogen centered radical during its metabolism involving oxidation (Alma Tostmann, et al, 2008). This free radical induced oxidative stress is one of the important mechanisms in isoniazid induced liver injury (Kale BP et al, 2003).

In the present study assessment of liver toxicity was done by measuring the levels of ALT, AST, ALP, GGT, BIL and ALB in serum. An elevated level of AST, ALT, ALP GGT and BIL in serum is an indication of hepatocellular disruption. When there is liver injury these enzymes leak into blood stream from the damaged tissues and show an elevated level in serum (Vinayak Dnyandev Sapakal et al, 2011). In this study, the elevated levels of these markers were seen after Isoniazid administration, thus confirming the hepatotoxicity (Group II). Co-administration of PAEE and TIEE with Isoniazid (Group III and IV) reversed the levels of these markers almost to normal. This shows that both PAEE and TIEE have a membrane stabilizing activity, there by preventing the disruption of hepatocytes by Isoniazid.

Decrease in albumin and total protein levels showed that administration of Isoniazid (Group II) has caused impairment of liver function by impairing the synthesizing capacity of liver parenchymal cells (Thapa BR, 2007). Co-administration of PAEE and TIEE with Isoniazid (Group III and IV) reversed the serum level of albumin to normal. This shows that both PAEE and TIEE have a protective action on liver parenchymal cells, there by preserving their synthesizing ability.

In this study, liver homogenate of the rats administered with Isoniazid showed a decreased activity of SOD & CAT and a decrease in GSH level. This gives a clear indication about the role of oxidative stress induced by Isoniazid. Antitubercular drugs induced oxidative stress was reported earlier (Vinayak Dnyandev Sapakal et al, 2011; Kale BP et al, 2003). Co-administration of PAEE and TIEE with Isoniazid (Group III and IV) has increased the SOD and CAT activity. They also increased the level of GSH in liver homogenate. This shows that both PAEE and TIEE have an antioxidant activity, there by preventing the cellular damage by reactive oxygen species.

Many hepatocellular biotransformation reactions are carried out by Cytochrome P-450 enzymes. This enzyme family mediates high-energy reactions leading to the generation of reactive metabolites of the drugs and their covalent binding to hepatic macromolecules and the formation of non functional adducts is one of the most accepted mechanism of drug induced liver injury. (William M. Lee, 2003; Vinayak Dnyandev Sapakal et al, 2011). One of the important mechanisms in isoniazid induced liver injury is believed to be accumulation of hydrazine, the toxic intermediate formed during its metabolism (Alma Tostmann et al, 2008). Both PAEE and TIEE might have blocked the conversion of Isoniazid to its toxic metabolite, or they might have rapidly converted the toxic metabolite to a non toxic form, thereby exerting their hepatoprotective action.

As mentioned earlier, the high energy metabolic reactions carried out by CYP-450 enzymes can lead to the covalent binding of drug to hepatocellular macromolecules, leading to the formation of nonfunctioning adducts. These large adducts migrate to cell surface of the hepatocytes, where they serve as target for immunological reactions mediated by cytolytic T cells and cytokines. This in turn will lead to apoptosis of liver (William M. Lee, 2003). PAEE and TIEE may exhibit liver protective action by inhibiting these immune mediated reactions. Previous studies have reported the immunomodulatory actions of these plants (Ganguly T et al, 2001; Oyewo et al, 2012).

Previous studies (Raja et al, 2011; Shyamjith M et al, 2012), have reported the presence of various phytoconstituents in the ethanolic extract of *Phyllanthus amarus* and *Tylophora indica*. From the above results it can be concluded that these phytoconstituents have a major role in the hepatoprotective action of these indigenous plants. Studies are ongoing to elucidate the exact role of each phytoconstituent in Isoniazid induced liver injury.

REFERENCES

- Alma Tostmann, et al. (2008). Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review. *Journal of Gastroenterology and Hepatology*; 23 :192–202.
- Bhattacharyya R, Bhattacharya S, Wenzel Mathers M, Buckwold VE (2003). *Phyllanthus amarus* root clone with significant activity against bovine viral diarrhoea virus – a surrogate model of hepatitis C virus. *Current Science* 84(4): 529-533.
- Bhattacharjee R, Sil PC. (2007). Protein isolate from the herb, *Phyllanthus niruri* L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties. *Food Chem Toxicol* 45(5):817-26.
- Beauchamp C and Fridovich I. (1971). Superoxide dismutase improved assay applicable to acryl amide gels. *Analytical biochemistry* 44:276-287.
- Bruce R, Todd JK, LeDune. (2001). Serum transaminase: its clinical use in diagnosis and prognosis. *BMJ* 1958:1125-1128.
- Directly observed treatment short course: Tuberculosis cure for all. *ICMR Bulletin*. <http://www.icmr.nic.in/bumar2001>.
- Doumas BT, Bayse DD, Carter RJ, Peters T Jr, Schaffer R. (1981). A candidate reference method for determination of total protein in serum. I. Development and validation. *Clin Chem*; 27:1642-50.
- Earl Hershfield. (1999). Tuberculosis Treatment. *CMAJ* 161 (4):405-411.
- Ganguly T, Sainis KB., (2001). "Inhibition of cellular immune responses by *Tylophora indica* in experimental models", *Phytomedicine, Proquest Health and Medical complete*, Vol. 8(5); 348-55.
- Global tuberculosis control: WHO report 2011.
- Goodwin JF., et al. (1965). Automation in Anal Chem Technical Symposia, 315-320.
- Goth L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinics Chimica Acta*; 196 : 143-152
- Harmanjit Kaur, Karanveer Singh. (2012). Brief phytopharmacological overview of *Tylophora indica* an endangered medicinal plant. *Int J Pharm Sci Res*; 3(11):4073-4076.

- Kale BP, Kothekar MA, Tayade HP, Jaju JB. (2003). Effect of aqueous extract of *Azadirachta indica* leaves on hepatotoxicity induced by antitubercular drugs in rats. *Indian J Pharmacol* 35:177-180
- Khalili H., Dashti-Khavidaki S., Rasoolinejad M., Rezaie L., Etminani M. (2009). Anti-tuberculosis drugs related hepatotoxicity; incidence, risk factors, pattern of changes in liver enzymes and outcome. *DARU*;17 (3) :163-167.
- Kong JM et al. (2003). Recent advances in traditional plant drugs and orchids. *Acta Pharmacol Sin* 24 (1): 7-21.
- Kumar Sunil, Sharma Priya. *Tylophora indica* an Indian ipecacuanha review. *International journal of phytotherapy research* 2012; 2(2):1-14.
- Luck H.(1974). *Methods in Enzymatic Analysis*. Academic Press (Ed. Bergmeyer), New York,:885.
- Malathi R, Ahamed John S and Cholarajan A. *Tylophora asthmatica* L. (2011). Prevents Lipid Peroxidation in Acetaminophen induced hepato toxicity in rats. *Asian J. Res. Pharm. Sci.* 1(3):71-73.
- Mark W Sonderup. (2011). Drug induced liver injuries. *CME*, 29(6):244-246.
- Mary Chatterjee, Parames Sil. (2006). Hepatoprotective effect of aqueous extract of *Phyllanthus niruri* on nimesulide induced oxidative stress *in vivo*. *Indian journal of biochemistry and Biophysics*;43:299-305.
- Mohamed Saleem TS, Madhusudhana Chetty C, Ramkanth S, Rajan VST, Mahesh Kumar K, Gauthaman K. (2010). Hepatoprotective Herbs – A Review. *Int. J. Res. Pharm. Sci.*; 1(1): 1-5.
- Mujeeb et al. (2009). Hepatoprotective activity of methanolic extract of *Tylophora indica* leaves. *International journal of Green pharmacy* 125-127.
- Naga Chalasani et al. (2008). Clinical advances in liver, pancreas, and biliary duct. *Gastroenterology*, 135:1924-1934.
- Narayan P Yadav et al. (2008). Synergistic effect of silymarin and standardized extract of *Phyllanthus amarus* against CCl₄-induced hepatotoxicity in *Rattus norvegicus*. *Phytomedicine* 15:1053–1061.
- OECD. (2000). Guidelines for the testing of chemicals revised draft guideline 423: acute oral toxicity. Paris: OECD;
- Oyewo, (2012). Emmanuel Bukoye, Akanji, Musbau Adewumi and Adekunle, Adeniran Sanmi. Immunomodulation Capabilities of Aqueous Leaf Extract of *Phyllanthus amarus* in male Wistar Rats. *Report and Opinion*, 4 : (1):22-37.
- Pauline Joseph et al. (2011). Outcome of standardized treatment for patients with MDR-TB from Tamil Nadu, India. *Indian J Med Res* ;,133:529-534.
- Patterson J, Swale J, Corra Maggs (1952). The estimation of serum Bilirubin. *Biochemical Journal* 52:100-105.
- Rajeshwar Y et al. (2008). In vitro lipid peroxidation inhibitory and antimicrobial activity of *Phyllanthus niruri* (Euphorbiaceae) Extract. *IJPT* 7: 67-70.
- Rashmi Mathur. (2011). Antimicrobial Effect of *Phyllanthus niruri* on Human Pathogenic Microorganisms. *Int. J. of Drug Discovery & Herbal Research*;1(4)238-238.
- Raja et al. (2011). Effect of *Phyllanthus niruri* Leaf Extract on Antioxidant Activity and UV Induced Chromosomal Aberration in Swiss Albino Mice. *Pharmacologyonline* 3: 169-174.
- Rosaiki SB., et al. (1970). *Ann.Clin.Biochem*;7:143.
- Sabitha Rani A, Sudeshna Patnaik, Sulakshanaand G ,Saidulu B. Review of *tylophora indica*- an antiasthmatic plant. *FS J Res Basic & App Sci* 2012 ;1(3):20-21.
- Sethuraman MG, Lalitha KG, Raj Kapoor B. (2003). Hepatoprotective activity of *Sarcostemma brevistigma* against CCl₄ induced hepatic damage in rats. *Current Science*;84(9):1186-1187.
- Sharma SM, Muralee Mohan, Suchetha Kumari, Shreyas J (2009). Sorake. Evaluation of Glutathione in oral squamous cell carcinoma. *Journal of Maxillofacial Oral Surgery* 8(3): 270-274.
- Shyamjith M , Anu Elizabeth Joy , Thyagaraju BM , Rao SN . (2012). Effect of *Tylophora indica* on Haloperidol Induced Catalepsy in Experimental Animal Models. *Drug Invention Today*, 4(12), 652-654
- Sude Eminzade, Fikriye Uras , Fikret V Izzettin. (2008). Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. *Nutrition & Metabolism* 5:1-8
- Tietz NW. (1983). Study group on alkaline phosphatase. A reference method for measurement of alkaline phosphatase activity in human serum. *Clini.Chem*; 29:751.
- Thapa BR ,Anuj Walia. Liver (2007). Function Tests and their Interpretation. *Indian Journal of Pediatrics* 74:663-671.
- Vratislav Chromy', Lenka S' va'chova', Luka' s' Novosa'd, Jiri' Jarkovsky', Pavel Sedla'k, Petr Hora' k, Hana Dobrovolna', Barbora Hlava' cova' . (2009). Albumin-based or albumin-linked calibrators cause a positive bias in serum proteins assayed by the biuret method. *Clin Chem Lab Med* 47(1):91–101

- Venkateswaran PS, Millman I ,Blumberg BS. (1987). Effects of an extract from *Phyllanthus niruri* on hepatitis B and woodchuck hepatitis viruses: In vitro and in vivo studies. *Proc. Natl. Acad. Sci. USA*;84:274-278.
- Vinayak Dnyandev Sapakal, Amol Bhalchandra Deore, Rahul Shivaji Adnaik,Tabassum Shikalgar, Nilofer S. Naikwade. (2011). Additive hepatoprotection of Ranitidine with Vitamin E in Rifampicin induced hepatotoxicity in rats. *Pharmacologyonline* 3: 20-33.
- Vipul B Gujarati, Nilesh J Patel, Venkat Rao N, Shivraj Gouda T and Md. Shalam. (2007). Hepatoprotective activity of alcoholic and aqueous extract of leaves of *Tylophora indica* in rats. *Indian J Pharmacol*; 39(1):43-47.
- WHO. (1993). .A review of current epidemiological data and estimation of future tuberculosis incidence and mortality. WHO/TB/93,173 Geneva WHO
- WHO (2012).Global tuberculosis report,
- Whitfield JB. (2001). Gammaglutamyltransfere.Critical reviews in clinical laboratory sciences 38(4): 263-355.
- William M. Lee. (2003). Drug-Induced Hepatotoxicity. *NEJM* 349(5): 474-485
- World Health Organization (WHO). (2009).Global tuberculosis 1. control 2009: epidemiology, strategy, financing. WHO/HTM/TB/2009.411. Geneva: WHO;