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The Hepato-protective effect of *phyllanthus longiflorus* Heyne Ex. Hook. F. in isoniazid and rifampicin induced hepatotoxicity in wistar rats

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Abstract

The present study was planned to evaluate the effect of ethanolic extract of *phyllanthus longiflorus* against isoniazid and rifampicin induced hepatotoxicity in wistar rats. In this study, we observed the hepatoprotective effect of *phyllanthus longiflorus* (EEPL) in Rifampicin (RIF) and Isoniazid (INH) induced hepatotoxicity in rats. A significant elevation was observed in the levels of serum AST, ALT, ALP and significant decrease level total protein and total albumin in G2 which received Rifampicin (RIF) and Isoniazid (INH) as compared to G1 rats who received normal saline. Elevated levels of these parameters in serum are presumptive markers of hepatotoxic lesions in the liver. On morphological examination in G4, 200mg/kg of *phyllanthus longiflorus* (EEPL) showed partial recovery in some liver. In this study Flavonoids in *phyllanthus longiflorus* (EEPL) might have a role in the recovery in Rifampicin (RIF) and Isoniazid (INH) induced hepatotoxicity in rats. The hepatoprotective role of *phyllanthus longiflorus* (EEPL) might be due to its antioxidant potential mechanism suggesting that the extract of plant may be useful to prevent the oxidative stress induced damage. More research is required in this View-point to develop a good hepatoprotective drug from *phyllanthus longiflorus* (EEPL) Purification of extracts and identification of the active principle may yield active Hepatoprotective ingredients.

Keywords: Hepatoprotective activity, *phyllanthus longiflorus*, isoniazid and rifampicin, male wistar rats.

Introduction

Isoniazid, although a highly effective drug in the treatment of tuberculosis, is well known for its hepatotoxicity Santhosh et al., 2006. The risk of severe hepatotoxicity caused by Isoniazid is approximately 1–2% of patients and 20% of patients are associated with liver enzyme elevations in plasma Sarich *et al*., 1999. Despite the undefined mechanism of Isoniazid hepatotoxicity, hydrazine and acetyl hydrazine are regarded as the main toxic metabolites of Isoniazid (Nelson *et al*., 1976, Timbrell and Wright 1979, Delaney and Timbrell 1995, Walubo *et al*., 1998). It is highly suggested that these two bioactive metabolites are produced by a series of enzymes including cytochrome P450 Sarich *et al*., 1999, Girling 1978 , Ono *et al.,* 1998) and could induce oxidative stress to cause hepatotoxicity Walubo *et al*., 1998).

In particular, CYP 2E1 is reportedly involved in Isoniazid-induced hepatotoxicity in humans Huang *et al*., 2003). Animals (Sarich *et al*., 1999, Yue *et al.,* 2004) microsomes (Nelson *et al*., 1976, Timbrell and Wright 1979) and HepG2 cells (Nicod *et al*., 1997). Since oxidative stress induced by CYP 2E1 was regarded as the major mechanism of Isoniazid hepatotoxicity, intracellular glutathione (GSH) enhancers and reactive oxygen species (ROS) scavengers were used as a potent anti-hepatotoxic drugs against Isoniazid toxicity. For example, 'thiol' compounds, such as *N*-acetylcysteine (NAC), effectively inhibited the Isoniazid hepatotoxicity in rat by supplying the intracellular GSH content (Attri *et al*., 2000).

Rifampicin (RIF), a powerful inducer of mixed-function oxidase, increases the hepatotoxicity of Isoniazid (INH) by enhancing the production of toxic metabolites from acetyl hydrazine (Ellard and Gammon 1976, Kalra *et al*., 2007). Rats show a similar genetically determined acetyl transferase activity as in humans and are more sensitive to Isoniazid (INH)-induced hepatotoxicity due to a high amidase activity, which results in release of large amount of acetyl hydrazine, which induces hepatotoxicity.

Anti-tubercular drugs mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation and source for destruction and damage to

the cell membrane (Georgieva *et al*., 2004). Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components have been reported in Rifampicin (RIF) and Isoniazid (INH) induced hepatoxicity (Tasduq *et al*., 2005).

Since all the drugs used in the treatment of tuberculosis are shown to have hepatotoxic effects, studies have been performed to prevent or reduce the toxicity by the use of natural herbal drugs and/or synthetic compounds, without interfering with the therapeutic action of the drugs. Garlic, Silymarin, N-acetylcysteine and several other herbal drugs are proved to have such effects. It is of importance to note that the inhibition of CYTP450 2E1 and antioxidant actions seem to be the common mechanism of action of herbal drugs (Sude *et al*., 2008). The purpose of the present study was to see the effect of *phyllanthus longiflorus* on INH and RIF induced hepatitis in wistar rats.

Materials and Methods

Plant material

The aerial part of *Phyllanthus longiflorus* Heyne Ex Hook.F. was collected from Courtallam hills (Tamil Nadu) at 1000m ht.

Preparation of plant extract for phytochemical screening and anti tumor activity

The leaves of the plant were dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction in a soxhlet apparatus using ethanol. The extract was subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure. The ethanolic extracts of the leaves were concentrated in a rotary evaporator. The concentrated ethanol extract were used for hepatoprotective studies.

Chemicals

Total albumin, Total protein, Aspartate transaminase (AST), Alanine Transaminase (ALT) AND Alkaline phosphatase (ALP) were assayed by using kits from Ranbaxy Diagnostic, New Delhi.

All the drugs, chemicals and reagents used for biochemical estimation were purchased from Sigma-Aldrich, USA.

Animals

Thirty adult male wistar rats weighing 200-250g were procured for this study. They were kept in the experimental research laboratory of K.M.College of pharmacy, Madurai. Rats were divided into 5groups, each having 6 animals. Before the commencement of the experiment, all animals were kept for one week under the same laboratory conditions, at a temperature of 22 ± 2^0C, relative humidity of 70 ± 4% and 12 hour light / dark cycle. They received nutritionally standard diet and tap water. The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals.

Induction of experimental hepatotoxicity

Rifampicin and Isoniazid solution were prepared separately in sterile distilled water. Rats were treated with Isoniazid (100 mg/kg, orally) and co-administered with rifampicin (100 mg/kg, orally) for 28 days (Yue *et al*., 2004, Saleem *et al*., 2008). In order to study the effect of Ethanolic extracts of *phyllanthus longiflorus* (EEPL) in rats, 200 and 200 mg/kg, orally were used. Silymarin (75 mg/kg orally.) was used as a standard drug in this study (Parthasarathy *et al*., 2007).

Treatment Protocol

Rats were divided into five groups each having six animals.

Group1 Served as normal control group received 10ml/kg normal saline.

Group2 Served as toxic control group received Rifampicin and Isoniazid 100mg/kg administered orally.

Group3 Served as standard group received Silymarin 75 mg/kg administered orally.

Group4 Served as extract (EEPL) treatment groups received 200 mg/kg administered orally.

Biochemical estimation

Rats were sacrificed 1 h after administration of drug on day 28.The blood was collected by retro-orbital artery puncture. Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. AST, ALT, ALP, Total Protein and albumin levels were estimated from the serum by using standard kits (Rajesh *et al.*,2005).

Histopathological studies

The liver was excised quickly and fixed in 10% formalin and stained with Hematoxylin and eosin and then observed under microscope for degeneration, fatty changes or necrotic changes as evidence of hepatotoxicity.

Statistical analysis

The results are expressed as mean ± SEM. The evaluation of the data was done using one way ANOVA followed by Newman – Keul's multiple range tests. Difference below $p<0.05$ implied significance.

Results

Effect of *phyllanthus longiflorus* (EEPL) on the serum AST levels

Results showed a significantly increased ($p<0.01$)level of serum AST in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly reduced ($p<0.01$) in rats of G3 and G4 treated with Silymarin and *phyllanthus longiflorus* (EEPL) *at a* dose of 200 and 200mg/kg..

Effect of *phyllanthus longiflorus* (EEPL) on the serum ALT levels

It is found a significantly increased ($p<0.01$) level of serum ALT in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly reduced ($p<0.01$) in rats of G3 and G4 treated with Silymarin and *phyllanthus longiflorus* (EEPL) *at a* dose of 200 and 200mg/kg..

Effect of *phyllanthus longiflorus* (EEPL) on the serum ALP levels

It is observed that Rifampicin (RIF) and Isoniazid (INH) caused significantly increased ($p<0.01$) level of serum ALP in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly reduced ($p<0.01$) in rats of G3 and G4 treated with Silymarin and *phyllanthus longiflorus* (EEPL) *at a* dose of 200 and 200mg/kg..

Effect of *phyllanthus longiflorus* (EEPL) on the serum total protein and total albumin levels

The results showed that Rifampicin (RIF) and Isoniazid (INH) caused significantly decreased ($p<0.05$) level of serum total protein and total albumin in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly increased ($p<0.01$) in rats of G3 and G4 treated with Silymarin and *phyllanthus longiflorus* (EEPL) *at a* dose of 200 and 200mg/kg.

Table. 1 Effect of *phyllanthus longiflorus* on serum enzymes

GROUPS	TOTAL PROTEIN (g/dl)	TOTAL ALBUMIN (g/dl)	AST (u/l)	ALT (u/l)	ALP (u/l)
G1	9.12±0.58	5.32±0.73	152.55±6.58	81.45±4.75	126.40±3.25
G2	4.64±0.32*a	2.50±0.35*a	250.40±8.85*a	165.25±5.65*a	261.40±6.35*a
G3	7.80±0.52*b	4.55±0.52*b	182.30±6.50*b	87.25±3.96*b	22.90±5.31*b
G4	6.75±0.48*b	3.80±0.50*b	215.55±7.55*b	114.15±4.46*b	243.45±5.52*b

All values are expressed as Mean ± SEM (n=6).

Values are expressed as Mean ± SEM.

Values were find out by using ONE WAY ANOVA followed by Newman Keul's multiple range tests.

* (a) values were significantly different from normal control at $p<0.01$.

* (b) Values were significantly different from toxic control at $p<0.01$.

Fig. 1 Effect of *phyllanthus longiflorus* (EEPL) on the serum total protein

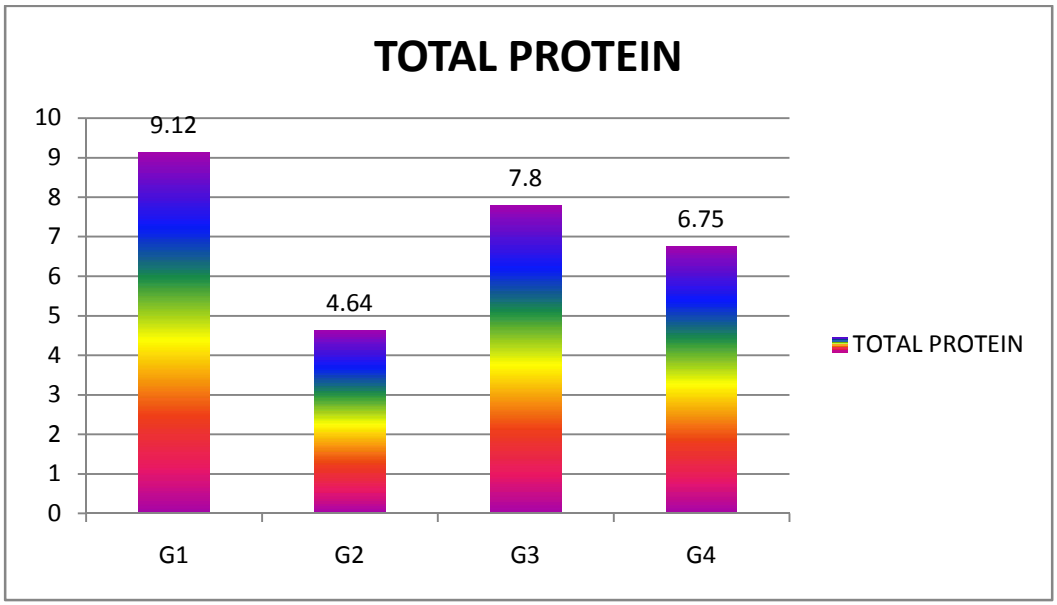


Fig. 2 Effect of *phyllanthus longiflorus* (EEPL) on the serum total albumin levels

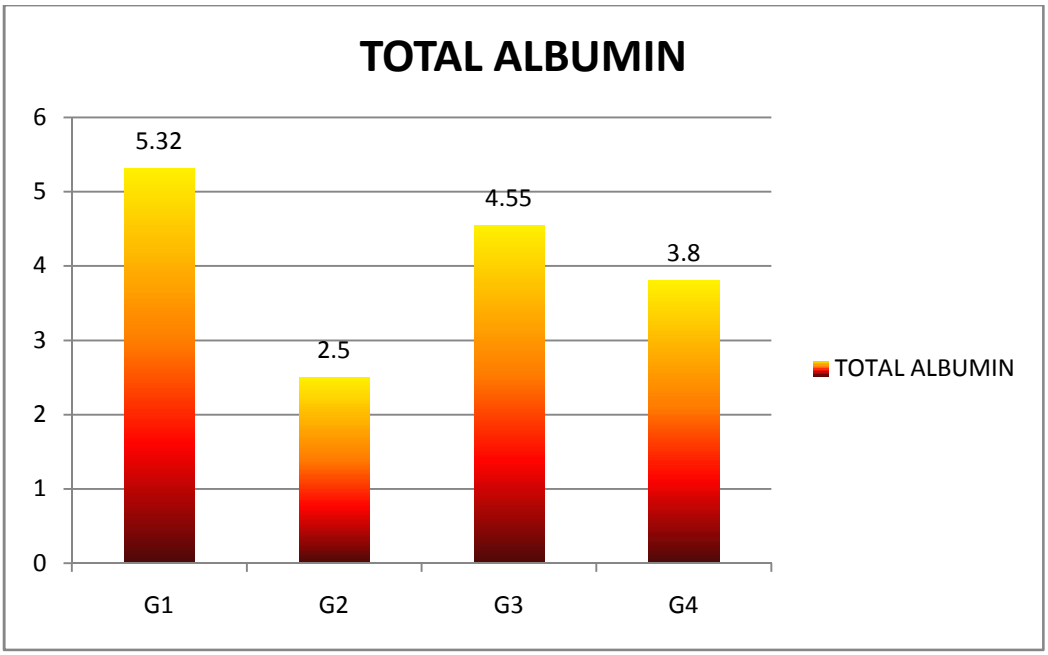


Fig. 3 Effect of *phyllanthus longiflorus* (EEPL) on the serum AST levels

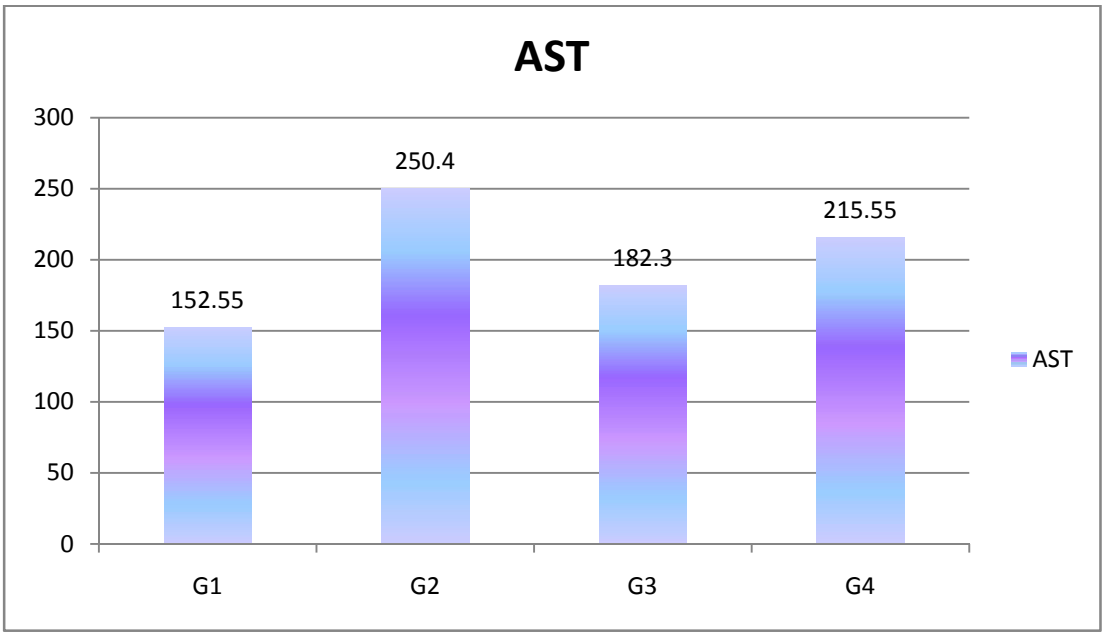


Fig. 4 Effect of *phyllanthus longiflorus* (EEPL) on the serum ALT levels

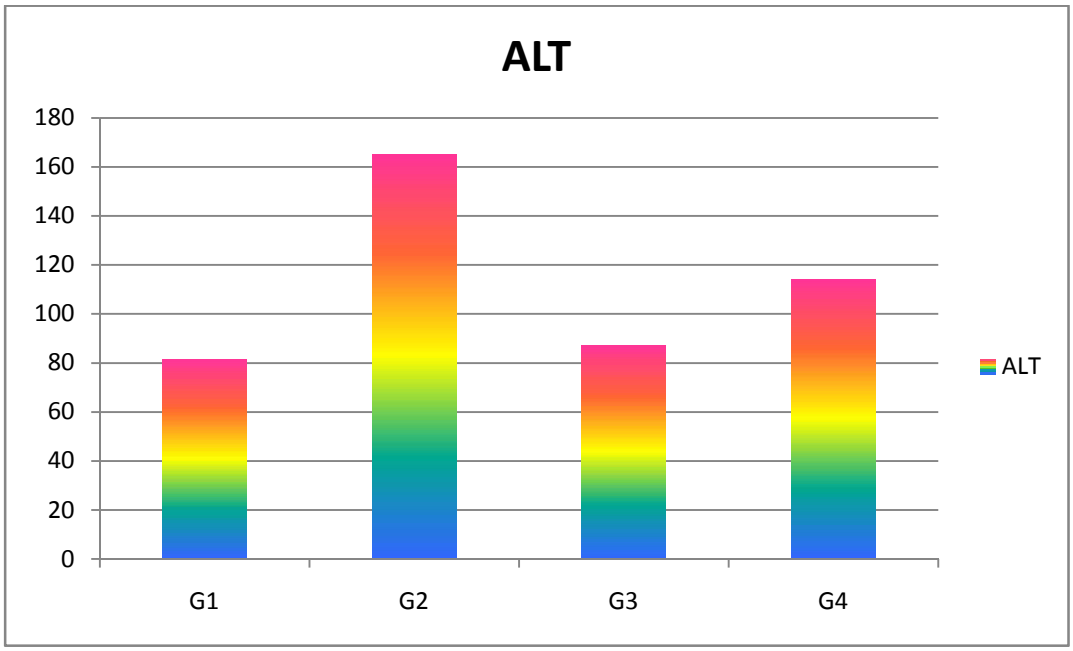
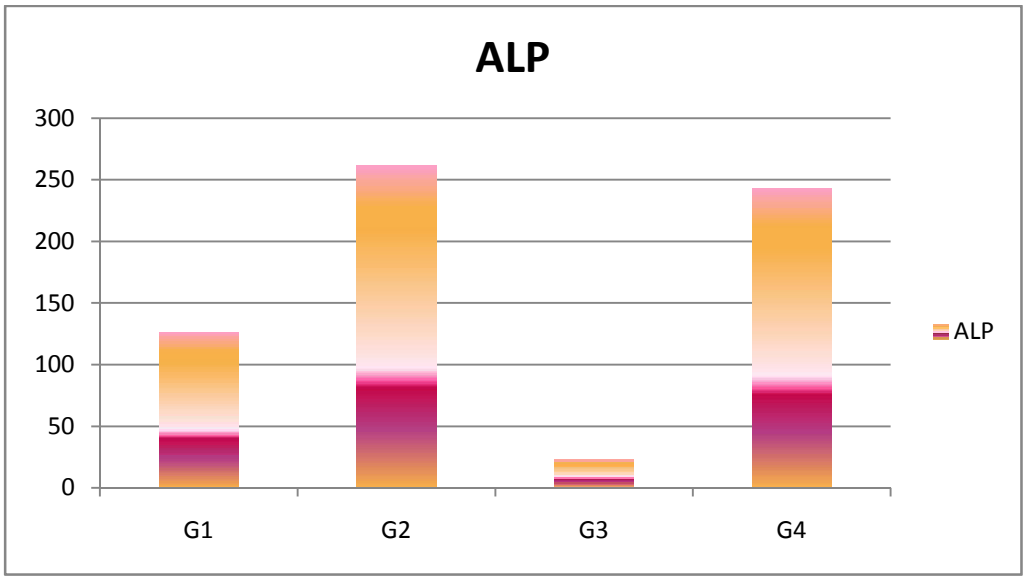


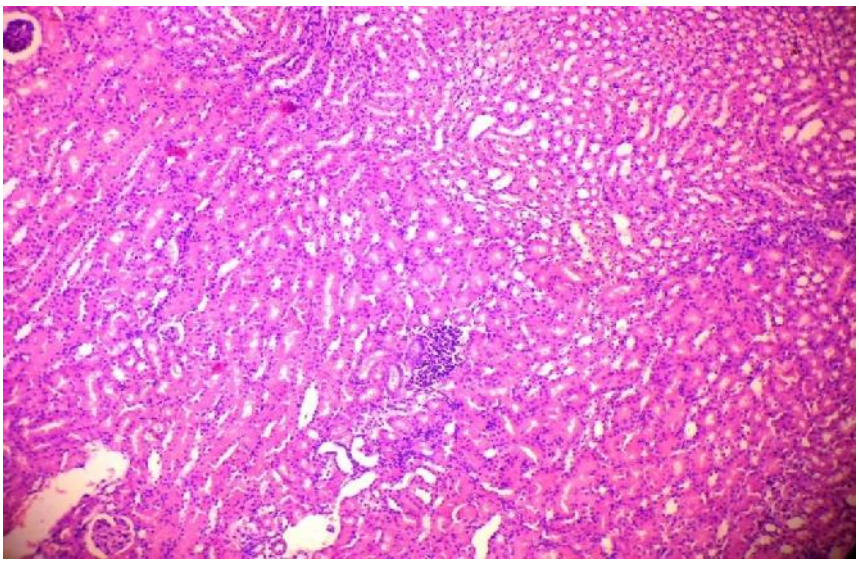
Fig. 5 Effect of *phyllanthus longiflorus* (EEPL) on the serum ALP levels



HISTOPATHOLOGY STUDY

Figure No: 1

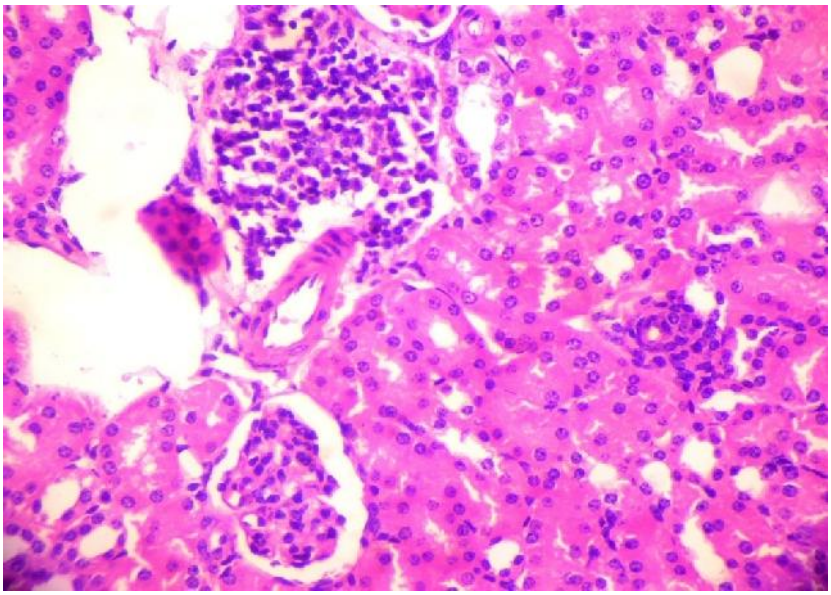
Normal Control Rat



Section of liver parenchyma with hepatocyte which appear normal, and central vein & portal tract are normal.

Figure No: 2

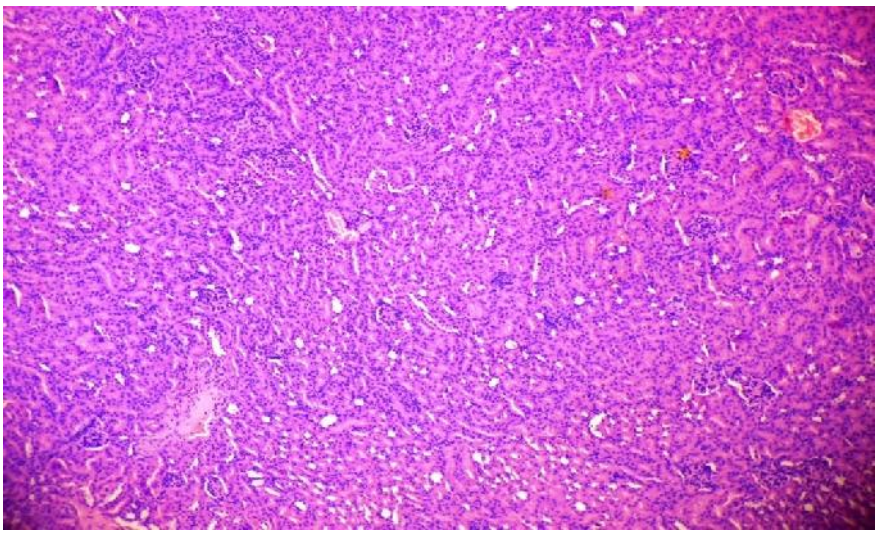
Toxic Control



Section of liver parenchyma with scattered focal area of necrosis of hepatocyte.

Figure No: 3

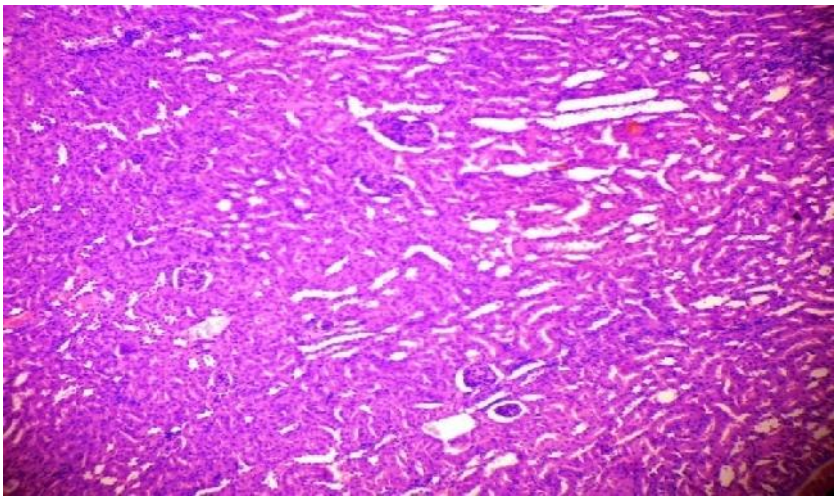
Positive Control



Section of liver parenchyma shows normal hepatic architecture

Figure No: 4

Treatment Control



Section of liver parenchyma shows normal hepatic architecture

Histopathological examination

In histopathological studies of liver, the control G1 showed normal gross appearance i.e. dark maroon color of liver having smooth surfaces, microscopically normal lobular appearance having normal central vein, radiating cords of heptocytes, normal portal tract in most of them (Figure 1). G2 rats that were given Rifampicin (RIF) and Isoniazid (INH), showed moderate to severe liver damage characterized by clear cytoplasm, vascular congestion, fatty changes, apoptosis and focal areas of necrosis and vacuolation of cytoplasm as a feature of ballooning degeneration (Figure 2). G3 Rats were given Rifampicin (RIF) and Isoniazid (INH) and Silymarin at a dose 75mg/kg showed normal liver architecture and occasional inflammatory cells with no traditis or necrosis (Figure 3). G4 Rats were given Rifampicin (RIF) and Isoniazid (INH) and dose of *phyllanthus longiflorus* (EEPL) (200 mg/kg) showed slight recovery and evidence of regeneration in some hepatocytes (Figure 4).

Discussion

Rifampicin (RIF) and Isoniazid (INH) are the most important first line drugs, used for the treatment of tuberculosis. Isoniazid (INH) can cause hepatotoxicity in 20% of patients and is usually associated with an inflammatory response (Tafazoli *et al*., 2008). Rifampicin (RIF) and Isoniazid (INH) are reported to induce hepatotoxicity judged by elevated serum AST, ALT, ALP and total bilirubin levels, presence of focal hepatocytic necrosis and portal triaditis (Pal *et al*., 2006). Plant-derived antioxidants such as Vitamin E, Vitamin C, polyphenol including phenolic acids, phenolic diterpenes, flavonoids, catechins, procyanidins, and anthocyanins are being increasingly suggested as important dietary factors. Supplementation with berry juice (Netzel *et al*., 2002) flavones from skullcap, catechins from green tea, anthocyanins from chokeberry, and condensed tannins from faba beans (Zdunczyk *et al.,* 2002) are indeed protective of oxidative stress indices in rats(Ramma *et al*., 2002).

The protective action of antioxidants is usually due to the inhibition of free radical chain reaction and the resultant prevention of peroxidative deterioration of structural lipids in membranous organelles. Circulating antioxidants mainly vitamin C and vitamin E and tissue enzymatic and non-enzymatic such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) play important role in alleviating tissue damage due to the formation of free radicals (Rajagopal *et al.,* 2003).

In this study, we observed the hepatoprotective effect of *phyllanthus longiflorus* (EEPL) in Rifampicin (RIF) and Isoniazid (INH) induced hepatotoxicity in rats. A significant elevation was observed in the levels of serum AST, ALT, ALP and significant decrease level total protein and total albumin in G2 which received Rifampicin (RIF) and Isoniazid (INH) as compared to G1 rats who received normal saline. Elevated levels of these parameters in serum are presumptive markers of hepatotoxic lesions in the liver.

Co-administration of Silymarin and dose of (200 & 200mg/kg) *phyllanthus longiflorus* (EEPL) ethanolic extract with INH and Rifampicin (RIF) and Isoniazid (INH) in G3 and G4 maintained the levels of AST, ALT, ALP, and serum total Protein and Total albumin towards normalcy as compared to G2 rats. This was most likely due to the anti oxidant effect of *phyllanthus*

longiflorus (EEPL) constituents. The results are in accordance with some previous studies (Pradeep *et al*.,2007, Pradeep *et al*.,2005). On morphological examination in G4, 200mg/kg of *phyllanthus longiflorus* (EEPL) showed partial recovery in some liver. In this study Flavonoids in *phyllanthus longiflorus* (EEPL) might have a role in the recovery in Rifampicin (RIF) and Isoniazid (INH) induced hepatotoxicity in rats.

Conclusion

This study showed that *phyllanthus longiflorus* (EEPL) has a significant protective action against the hepatotoxicity induced by the drugs used in the treatment of tuberculosis. The hepatoprotective role of *phyllanthus longiflorus* (EEPL) might be due to its antioxidant potential mechanism suggesting that the extract of plant may be useful to prevent the oxidative stress induced damage. More research is required in this View-point to develop a good hepatoprotective drug from *phyllanthus longiflorus* (EEPL) Purification of extracts and identification of the active principle may yield active Hepatoprotective ingredients.

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