

HEPATO PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF PIPER LONGUM AND PIPERINE WHEN ADMINISTERED WITH ANTITUBERCULAR DRUGS

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ABSTRACT

The objective of the study was to assess the hepatoprotective effect of aqueous extract of *Piper longum* and piperine when administered with normal dosage of antitubercular drugs. The parameters assessed were lipid peroxidation and levels of reduced glutathione in liver homogenate. Histopathological studies of liver was also carried out. Reduced glutathione levels were significantly decreased ($p < 0.001$) and lipid peroxidative products levels were significantly increased ($p < 0.001$) in the group treated with antitubercular drugs compared to controls. Administration of aqueous extract of *Piper longum* or piperine with antitubercular drugs significantly increased the reduced glutathione levels ($p < 0.001$) and decreased lipid peroxidation ($p < 0.001$). Histopathological studies indicate no significant change between the control and the group administered with antitubercular drugs. Even in studies carried out with *Piper longum* extract in infected animals, administration of antitubercular drugs significantly increased lipid peroxidation ($p < 0.001$) and decreased reduced glutathione levels ($p < 0.05$). Administration of aqueous extract of *Piper longum* with antitubercular drugs significantly increased the reduced glutathione levels ($p < 0.001$) and decreased lipid peroxidation ($p < 0.001$). The results indicate that administration of anti TB drugs in normal doses for a period of 45 days does not cause significant histopathological changes in liver but decreases the reduced glutathione levels and increases lipid peroxidation. This change in antioxidant status is prevented on administration of *Piper longum* aqueous extract and piperine with antitubercular drugs, thus proving their hepatoprotective effect.

INTRODUCTION

Plants have gained attention for their therapeutic value due to their antimicrobial activity and hepatoprotective potential. The ripe fruit of *Piper longum* has been widely used as popular medicine to treat diseases like tuberculosis and leprosy (Kirtikar *et al.*, 1987). The hepatoprotective effect of *Piper longum* in carbon tetrachloride induced liver damage has been studied. (Rege *et al.*, 1984) The liver protective potential of piperine has also been evaluated. (Koul *et al.*, 1993). Anti tubercular drugs namely Isoniazid, Rifampicin and Pyrazinamide used in short term chemotherapy are known to be potentially hepatotoxic. (Parthasarathy *et al.*, 1986). Experiments with experimental animals show that antitubercular drugs administered in toxic doses affect the liver and its membrane and other organelles. (Skakun and Jabachuk 1992a).

Hence, aqueous extract of fruits of *Piper longum* and piperine were chosen to study their hepatoprotective potential on administration with normal doses of antitubercular drugs.

MATERIALS AND METHODS

The antitubercular drugs Isoniazid, Rifampicin and Pyrazinamide were purchased from Sigma Chemical Company (USA) and piperine was obtained from Sami Labs, Bangalore.

Swiss albino mice weighing around 25g were housed in well designed animal house in large spacious cages and were given food and water *ad libitum* during the course of the experiment. The animals were divided into four groups of five animals each (males 2, females 3). The group I served as normal control. The group II was administered anti TB drugs-Ethambutol (25mg/kg body wt), Rifampicin (12mg/kg body wt), Isoniazid (10mg/kg body wt) and Pyrazinamide (35mg/kg body wt). Group III was administered anti TB drugs with aqueous extract of *Piper longum* fruit (0.5g/kg body wt). Group IV was administered anti TB drugs with Piperine (15mg/kg body wt). The drugs were administered in the form of a fine suspension with a feeding tube. The drugs were administered for 45 days and the animals were sacrificed. The liver was dissected and washed in ice cold saline. A section of the liver was used for histopathological examination (Photo 1 and Photo 2). A homogenate was prepared in 0.1M Tris buffer of pH 7.4 (20% solution) and used for estimation of lipid peroxides (Okhawa *et al.*, 1979) and reduced glutathione (Moron. *et al.*, 1979). Blood was collected and level of lipid peroxides are estimated in the serum sample. Protein was estimated in liver homogenate by the method of Lowry *et al.* (1951).

For studies with infected animals, the animals were divided into three groups of five animals each (males - 3, females - 2).

The groups were infected with H37Rv by a tail vein injection of the suspension after counting using Arnold .R.Horwell Thoma counter. After the infection was established (two weeks) group I served as control. Group II was administered anti TB drugs (as mentioned above). Group III was administered anti TB drugs with *Piper longum* fruit extract (0.5g/kg body wt). After fifteen days, the animals were sacrificed. Reduced glutathione and lipid peroxidative products level were estimated in the liver homogenate. Statistical analysis was carried out using student's t test.

RESULTS

The results of the study with uninfected animals (Table 1) show that lipid peroxides in liver homogenate as well as serum are significantly high in the group treated with anti tubercular drugs compared to normal control ($p < 0.001$). On administration of the aqueous extract of fruits of *Piper longum* along with the anti TB drugs, the levels of lipid peroxides in liver homogenate as well as serum are significantly decreased ($p < 0.001$) compared to the group treated with antitubercular drugs. Similarly on administration of piperine along with anti TB drugs, lipid peroxides in serum and in liver homogenate are significantly reduced ($p < 0.001$) compared to the group treated with antitubercular drugs. The levels of reduced glutathione are also significantly reduced ($p < 0.001$) in the

group treated with anti tubercular drugs compared to normal control. (Table1). Administration of extract of fruits of *Piper longum* and piperine along with the anti TB drugs significantly increased the levels of reduced glutathione ($p < 0.001$) Histopathological studies indicate no significant change between the control and the group administered with antitubercular drugs. On extending the study of hepatoprotective effect to infected animals (Table 2), it was again observed that there was a significant increase ($p < 0.001$) in lipid peroxides in liver homogenate of animals on administration of anti TB drugs. The levels were lowered significantly ($p < 0.001$) on administration of extract of fruits of *Piper longum* with anti TB drugs compared to the group treated with antitubercular drugs. There was a significant decrease ($p < 0.05$) in reduced glutathione levels in the group treated with anti TB drugs. On administration of extract of fruits of *Piper longum* with anti TB drugs a significant increase ($p < 0.001$) in the levels of reduced glutathione is observed compared to the group treated with antitubercular drugs.

DISCUSSION

Suppression of antioxidant system in anti-tubercular drug treated rats has been reported (Skakun *et al.*, 1992b) Isoniazid is known to deplete glutathione stores and lead to subsequent hepatocellular damage (Crippin 1993). Decreased glutathione levels observed in group treated with antitubercular drugs

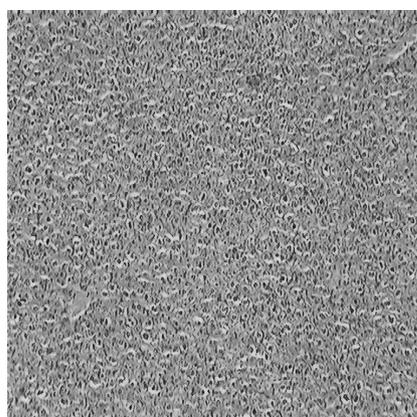


Photo 1: Liver of control group (HE x 100)

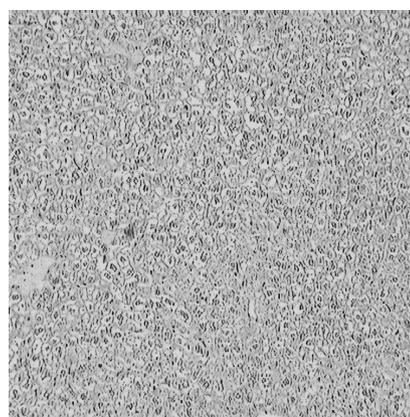


Photo 2: Liver of group treated with antitubercular drugs (HE x 100)

Table 1: Level of lipid peroxides and reduced glutathione in experimental groups (uninfected)

Particulars	I Normal control	II Antitubercular drugs	III Antitubercular drugs and PL extract	IV Antitubercular drugs and piperine
LPO (in homogenate) as nanomoles of MDA/g protein	72.74 ± 1.40	91.63 ± 2.00 ***	72.68 ± 1.91 a NS b ***	70.60 ± 0.45 a * b ***
LPO (in serum) in μmoles of MDA/ Lt.	2.90 ± 0.10	4.24 ± 0.07 ***	2.94 ± 0.06 a NS b ***	2.81 ± 0.06 a NS b ***
GSH (μg/g liver)	50.87 ± 1.07	15.34 ± 0.67 ***	38.00 ± 0.80 a *** b ***	44.35 ± 1.58 a *** b ***

The values represent Mean ± SD of five animals; the symbols represent statistical significance. a = compared with control, b = compared with group II; * - $p < 0.05$, *** - $p < 0.001$, NS - Not significant

Table 2: Level of lipid peroxides and reduced glutathione in experimental groups (infected animals)

Particulars	I Control	II Antituberculous drugs	III Antituberculous drugs and PL extract
LPO (in homogenate) as nanomoles of MDA/g protein	123.39 ± 1.97	138.61 ± 3.45 ***	103.72 ± 2.72 a *** b ***
GSH (μg/g liver)	23.25 ± 1.35	20.97 ± 0.53 *	54.04 ± 0.75 a *** b ***

The values represent Mean ± SD of five animals; the symbols represent statistical significance. a = compared with control, b = compared with group II; * - $p < 0.05$, *** - $p < 0.001$, NS - Not Significant

may also be due to its increased utilization in protecting thiol group containing proteins from lipid peroxides.

Hepatoprotective potential of compounds has been evaluated based on their ability to decrease lipid peroxidation and increase the levels of reduced glutathione. Such results have been reported in earlier studies while evaluating the hepatoprotective effect of Liv 100 (Saraswathy *et al.*, 1998). *Moringa Oleifera* (Pari *et al.*, 2002), tocopherol acetate (Skakun *et al.*, 1992b). *Solanum Xanthocarpum* (Talib Hussain *et al.*, 2012) and silymarin (Sandeep Bansal *et al.*, 2007) when administered with anti TB drugs. In an earlier study of the hepatoprotective effect of *Piper longum* on administration with anti TB drugs level of lipid peroxides and reduced glutathione etc was not evaluated. (Chhajed. *et al* 1991). Hence, this study was planned with aqueous extract of *Piper longum* and piperine. In this study, hepatoprotective effect of *Piper longum* and piperine on administration with the anti TB drugs, is demonstrated since it lowers lipid peroxidation and also increases reduced glutathione levels as observed in previous studies. In the experimental period though no histopathological studies were observed in the group treated with antitubercular drugs compared to control, high levels of lipid peroxides observed in groups treated with antitubercular drugs may lead to various deleterious effects including tissue damage and necrosis as reported (Tappel. 1980). In this study it is observed that administration of *Piper longum* and piperine with anti TB drugs lowers lipid peroxidation and thus exerts hepatoprotective effect.

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