Discussion
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ELUCIDATION OF STRUCTURES OF COMPOUNDS Y AND P

Compound P isolated from the acetone extract has a structure similar to that of piperine as concluded from UV, IR and NMR studies based on the peaks obtained for characteristic groups. (TABLES 1 and 2 (FIGURES 1-5)

![Structure of Piperine](image)

Structure of Piperine

It is believed that compound Y may have a closely related molecular structure to compound P based on the following observations.

The UV spectra of piperine and compound P show an absorption maxima at 340 nm. Compound Y does not have an absorption maxima at 340nm. This indicates the absence of an extended conjugated system, which means that N-C linkage may be absent. This is further
confirmed by a weak amide bond stretch in IR spectra. The absence of $N\overset{\text{CH}_2}{\text{CH}_2}$ peak in NMR spectra also confirms this observation. A C-C bond may be present in compound Y instead of a N-C bond (between C12 and nitrogen) present in compound P.

**IN VITRO STUDIES**

(A) **ANTIBACTERIAL ACTIVITY**

All the basic compounds precipitated from the aqueous extract as WP exhibits anti bacterial and anti mycobacterial activity only at very high concentrations as compared to the reference drug. (TABLE 3) The minimum inhibitory concentration is very high compared to that of the standard drug.

Results of disc diffusion indicate that compounds Y and P extracted from fruits of *Piper longum* do not possess significant antibacterial and anti-mycobacterial activity at low concentrations as compared to the reference drug. (TABLE 4). (PHOTOS 2 and 3)

Previous studies have also so far reported that the essential oil and amides of *Piper longum* possess significant antimycobacterial activity (Bhargava and Chauhan.,1968),(Gupta *et al.*, 1980)
(B) BIOENHANCER STUDY

The aqueous extract of fruits of *Piper longum* does not act as a bio-enhancer *invitro* along with drug Ofloxacin (tested against *E. coli*) (TABLE 5) Rifampicin (tested against *S.aureus*) (TABLE 6) (PHOTO 4a and 4b) and Ethambutol, Isoniazid (tested against *M. smegmatis*) (TABLES 7 AND 8) as concluded from the disc diffusion studies. For the drug alone compared with drug and *Piper longum* fruit extract there is no significant change in the diameter of zone of inhibition. Piperine has been reported to act as a bio enhancer in vitro with rifampicin (Veena Balakrishnan *et al.*, 2001). But no such effect is observed with aqueous extract of fruits of *Piper longum*.

STUDIES WITH UNINFECTED ANIMALS

The results of table 9 show that there is no significant change in the levels of liver enzymes in the homogenate. This is in accordance with the observation that no significant histopathological changes in the liver were noted in the groups. Decrease in the levels of marker enzymes in liver is usually associated with leakage due to tissue injury which is not evident in this case.
Though there is no evidence of tissue damage, the results of the study (table 10) 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. On administration of the extract of fruits of *Piper longum* along with the anti TB drugs, the level of lipid peroxides in liver homogenate as well as serum is similar to the control group. On administration of piperine along with anti TB drugs lipid peroxides in serum is similar to the control group and in liver homogenate it is even reduced compared to the control group. In the case of groups treated with extract of fruits of *Piper longum* and piperine alone, the level of lipid peroxides in liver homogenate is reduced compared to normal control. Since high levels of lipid peroxides is reported to be associated with various deleterious effects including tissue damage and necrosis, it is concluded that administration of the extract of fruits of *Piper longum* along with the anti TB drugs exerts a hepatoprotective effect.

The level of reduced glutathione is also significantly reduced in the group treated with anti tubercular drugs compared to normal control. Administration of extract of fruits of *Piper longum* and piperine along with the anti TB drugs increases the level of reduced glutathione. The levels of reduced glutathione in the groups that were
administered extract of fruits of *Piper longum* and piperine alone were similar to that of control group. Decreased glutathione levels may be due to its increased utilization in protecting thiol group containing proteins from lipid peroxides. Therefore, increase in levels of reduced glutathione on administration of extract of fruits of *Piper longum* and piperine indicate very good protection to liver.

Similar results have been reported by S.D Saraswathy *et al.*, 1998, while evaluating the hepatoprotective effect of Liv 100 and Skakun.N.P and Tabachuk.O.P., 1992 while evaluating the hepatoprotective effect of tocopherol acetate.

The hepatoprotective effect of piperine has been demonstrated in carbon tetra chloride induced liver damage(Koul.I.B and Kapil.A., 1993). The hepatoprotective effect of *Piper longum* on administration with anti TB drugs has been evaluated previously (Chhajed *et al.*, 1991). In the study the dosage of drugs used was high and therefore histopathological changes were observed. Level of lipid peroxides, reduced glutathione *etc* was not evaluated. In the present study normal dosage of drugs was used, therefore no histopathological changes were observed. The hepatoprotective effect has been demonstrated by evaluating level of lipid peroxides and reduced glutathione.
EXPERIMENTS WITH INFECTED ANIMALS

On extending the study of hepatoprotective effect to infected animals (table 11), it is again observed that there is a significant increase in lipid peroxides in liver homogenate of infected animals which rises still further on administration of anti TB drugs. The levels are lowered on administration of extract of fruits of *Piper longum* with anti TB drugs.

It is again observed that there is a significant decrease in reduced glutathione levels in the infected animals. The levels are still less in the infected group treated with drugs. On administration of extract of fruits of *Piper longum* with anti TB drugs an increase in the levels of reduced glutathione is observed. In the group administered with extract of fruits of *Piper longum* alone there is a significant increase compared to the control group which confirms the hepatoprotective effect.

Even in the study with infected animals, no histopathological changes in liver was observed in the different groups. Therefore, a decrease in level of liver enzymes ALP and ALT (table 12) in infected animals and the decrease in the levels of all the three enzymes in the infected group treated with drugs may be attributed to the accumulation of the oxidized product of glutathione which results in
activation of enzymes containing thiol group and inhibition of protein synthesis (Lil.J.L et al., 1988).

**BIO AVAILABILITY STUDY IN HEALTHY HUMAN VOLUNTEERS**

It is evident from table 13 and table 14 that the bioavailability of pyrazinamide and rifampicin is greater in the group in which fruits of *Piper longum* was administered with anti TB drugs. The peak concentrations of these two drugs is also increased in the group in which fruits of *Piper longum* was administered with anti TB drugs. This is further substantiated by decreased urinary excretion of these drugs. (table 16). But no significant change has been observed in the time of peak concentration. Increased bioavailability of these drugs with piperine has been reported earlier. (Kapil.R.S et al., 1995) Qazi et al., 2003). The increase in peak concentration of pyrazinamide on administration with fruits of *Piper longum* is similar to that observed with administration of piperine as reported where as the increase in peak concentration of rifampicin on administration with fruits of *Piper longum* is not as high as reported with administration of piperine.

Though there is a decrease in peak concentration and sixth hour concentration of isoniazid in the group administered with *Piper longum* fruits with anti TB drugs, there is no significant change in the
bioavailability of the drug as evidenced from AUC value. (table 15) This is in accordance with the fact that there is no significant change in the urinary excretion of the drug. (table 16). Though an increase in bioavailability of isoniazid has been reported when administered with piperine, decreased bioavailability of isoniazid when administered with Trikatu (Piper longum, Piper nigum and Zingiber officinale) has also been reported (R.S.Karan et al., 1998). In the present study on administration of Piper longum fruits alone with anti TB drugs, no significant change in bioavailability was noted.

EFFECT OF ADMINISTRATION OF FRUITS OF PIPER LONGUM IN HEALTHY HUMAN VOLUNTEERS

The results also point out that administration of 0.5g of fruits of Piper longum for 40 days does not adversely affect the various biochemical and hematological parameters in healthy human volunteers. (table 17, 18 and 19). This assures safety in the use of Piper longum fruits. Previous studies carried out in animals report no change in hematological parameters on administration of Piper longum (Shah et al., 1998). The biochemical parameters were not evaluated.