

## Effect of Piper Nigrum (L.) on Hepatotoxicity Induced by Ethionamide and Para Amino Salicylic Acid in Sprague- Dawley Rats

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Fresh seeds of *Piper nigrum* were procured from the botanical garden of KokanKrushiVidyapeeth, Dapoli, Ratnagiri. The ethanolic extract of the seeds was carried out by soxhlate extraction method. Sixty four (64) Sprague- Dawley rats (average weight 150 - 240 g) of each sex were used for the experiment. The drugs ETH and PAS drug and *Piper nigrum* were given to respective groups daily for 28 days. At the end of study various biochemical parameters were analyzed from serum such as, ACP, ALP, SGOT (AST), SGPT (ALT), Bilirubin, Total Cholesterol, HDL Cholesterol, and TGL. Liver tissues were analyzed for Histopathology. The data was statistically analyzed by one way analysis of variance (ANOVA). The value  $p < 0.05$  considered as significant. Administration ETH and PAS in Sprague-Dawley rats showed hepatotoxicity in test groups which was confirmed by biochemical parameters and histoarchitecture examination of liver. Ethanolic extract of *Piper nigrum* (Linn.) seeds were administered to the test groups have ameliorated the toxic effect of the drugs. It is concluded that the *Piper nigrum* (Linn.) acts as hepato-protective agent hepato-toxicity caused by Ethionamide and Para amino salicylic acid in *Sprague-Dawley* rats .

**Keywords:** *Piper nigrum*, ETH ,PAS, histoarchitecture, liver. rats.

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism<sup>1</sup>. Liver disease is still a worldwide health problem. Drug-induced hepatotoxicity is one of the major concerns which limit the therapy and drug use. About 2% of all causes of jaundice in hospitalized patients are drug induced. Approximately quarter of cases of fulminant hepatic failure are thought to be drug related. More than 900 drugs have been implicated in causing

liver injury and it is the most common reason for a drug to be withdrawn from the market<sup>2</sup>

Tuberculosis (TB) is transmittable diseases caused by *Mycobacterium tuberculosis*. Despite medical advancement tuberculosis is very lethal and is the main cause of the human deaths in many countries. Every second someone in the world is infected with tuberculosis, with an estimated 9.6 million new cases each year. Approximately a third of the world's population is currently infected with tuberculosis and up to 10% of these will go to develop active TB, leading to 1.6 million deaths per year<sup>3</sup>. It is studied that

the development of MDR-TB is due to misuse of proper antibiotic treatment by patients and unfocused physician observations to these patients. Due to very modern issue associated with MDR-TB the Second line Tuberculosis drugs are used.

Ethionamide (eth eye on a mide) is most frequent drug used which shares the similarities with the Isonizid in terms of structure and antimycobacterial function. The daily oral dose of Ethionamide is 250 mg/kg and can be given up to 1 gm if well tolerated by patients. In the some cases of hepatotoxicity induced by ethionamide have been serious and malefic cases are also reported<sup>4</sup>.

Para-amino salicylic acid (PAS) was the first antibiotic found to be efficient in the treatment of tuberculosis in the 1940s<sup>5</sup>. PAS treatment is uncommon and a highly drug resistant strain seems to have limited resistance to this drug. Thus, PAS became the principle second line agent for the treatment of MDR-TB<sup>6</sup>. PAS may cause the hepatitis (Prasad et. al., 2006). Hepatotoxicity is one of the most frequent and serious adverse effects of anti-TB medications and may reduce treatment effectiveness by compromising treatment regimens<sup>7,8</sup>.

A lot of medicinal plants, traditionally used for thousands of years, are present in group of herbal preparation of the Indian traditional health care system. Today, about 80% of the world population dependent on botanical agents as medicine to meet their health issues<sup>9</sup>. In developing countries, the traditional plant remedies are widely used to treat various ailments. Many varieties of plants have been used for treating different kinds of diseases including hepatoprotective potentials<sup>10</sup>. Now days the dietary supplements and herbal remedies have increase the interest of researchers to treat different kind of diseases. In India, over 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used<sup>11,12</sup>.

*Piper nigrum* (Linn.) (familyPiperaceae) is one of the most commonly used spices and considered as "The King of spices" among various spices. *Piper nigrum* is effective anti-M. Tuberculosis and is active against both drug sensitive and resistant strains of TB<sup>13</sup>. *Piper nigrum* along with other phytoconstituents contains major pungent alkaloid Piperine which is known to possess many interesting pharmacological actions. Piperine has been found to enhance the therapeutic

efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolizing enzymes<sup>14</sup>. It is also known to enhance hepato-protective action<sup>15</sup>. In view of severe undesirable side effects of synthetic drugs, there is a need to focus on systematic research methodology and to study the scientific basis for the traditional herbal medicines that are claimed to show hepato-protective potential.

Therefore, in view of the above literature survey it was found that ETH and PAS used as antituberculosis drugs but at the same time these drugs are responsible to caused hepatotoxicity in the human beings. Therefore, in the present study, attention has been given to find the effect of *Piper nigrum* on hepatotoxicity induced by ETH and PAS in the Sprague-Dawley rats.

## MATERIALS AND METHODS

### Collection of sample

Fresh seeds of *Piper nigrum* were procured from the botanical garden of KokanKrushiVidyapeeth, Dapoli, Ratnagiri. The initial identification was done by referring related literature and final identification and confirmation was done at the department of horticulture, KokanKrushiVidyapeeth, Dapoli, Ratnagiri prior to process the sample at the department of Zoology S.S & L.S. Patkar College Goregaon (west), Mumbai India.

### Extraction

The ethanolic extract of the seeds was carried out by soxhlate extraction method. The sample was evaporated to dryness and powder was weighed and the yield so obtained was collected in a sterile container and kept at -20 0C till further use. The weight of the powder was calculated based on weight of the seeds.

### Purchas of drugs

The drugs ETH (Macleods Pharmaceuticals Ltd) and PAS (Lupin Ltd) were purchased following the Prescription of Physician from B.J. Medical College and Sassoon General Hospital, Pune, Maharashtra.

### Experimental Design

Sixty six(64) Sprague- Dawley rats (average weight 150 - 240 g) of each sex were used for the experiment. They were purchased and procured from the National Toxicological

Centre, APT Testing & Research Pvt. Ltd. (ATR) Pune. The experimental study was approved by Ethical committee at APT Research Foundation, Pune prior to the experimentation (CPCSEA NO. 40/PO/ReBiRc/S/99/. 11. 03. 2014). The animals were acclimatized, maintained and housed in APT laboratory for a week. The controlled humidity and temperature at 24°C; humidity, 12-hlight/12 hrs dark cycle was also maintained by feeding the rats with commercial rat pellets and water available ad libitum.

#### Biochemical assay

Blood samples of the above groups were taken after 28th day by heart puncture for estimation of liver functional test. Assessment of liver damage were done by biochemical investigations of Serum glutamic-pyruvic transaminase (SGPT) and Serum glutamic-oxaloacetic transaminase (SGOT) by <sup>16</sup>Serum bilirubin (Bil) by<sup>17</sup> and Serum alkaline phosphatase (ALP) by <sup>18</sup>Cholesterol and HDL High density lipid by<sup>19</sup>Triglyceride by<sup>20</sup>, Acid Phosphatase by<sup>21</sup>.

#### Histopathological analysis

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol

(50–100%), cleared in xylene, and embedded in paraffin. Five micron thick sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty degenerative changes, hyaline regeneration, ballooning degeneration as proposed by<sup>22</sup>, and histological structure of liver tissue were examined under the Biological digital microscope-Motic B1 Series.

#### Administration of Test Article

The test article at the above concentration was administered to each rat by a single oral gavage. The animals were dosed using a stainless steel intubation needle fitted onto a suitably graduated syringe. The dosage volume administered to individual rat was adjusted according to its most recently recorded body weight. Animal weights were determined weekly along with food consumption.

Animals were randomly divided into following groups containing 8 animals (4 males and 4 females) in each group.

Test drug and inducers were given to respective groups as indicated in the table daily for 28 days. At the end of study various biochemical

Groups	Specification	Treatment specifications
1	Normal control	Animals fed with rat pellets and ordinary water
2	PnS	PnS (500 mg/kg bw)
3	ETH	ETH (132 mg/kg bw)
4	PAS	PAS (400 mg/kg bw)
5	ETH + PAS	ETH (132 mg/kg bw) + PAS (400 mg/kg bw)
6	ETH + PnS	ETH (132 mg/kg bw) + PnS (500 mg/kg bw)
7	PAS + PnS	PAS (400 mg/kg bw) + PnS (500 mg/kg bw)
8	ETH + PAS + Pns	ETH (132 mg/kg bw) + PAS (400 mg/kg bw)+Pns (500 mg/kg bw)

\*ETH=Ethionamide, PAS=Para amino salicylic acid, PnS= *Piper nigrum* Linn. Seeds ethanol extract

parameters were analyzed from serum such as, ACP, ALP, SGOT (AST), SGPT (ALT), Bilirubin, Total Cholesterol, HDL Cholesterol, and TGL. Liver tissues were analyzed for Histopathology of Liver by using method as proposed by Davidson; 1979

#### Statistical analysis

The data was statistically analyzed by one way analysis of variance (ANOVA). The value  $p < 0.05$  considered as significant.

#### RESULTS AND DISCUSSIONS:

The mean concentration of Serum Biochemical investigations of Serum ACP: Serum Acid Phosphatase, ALP : Serum Alkaline Phosphatase, SGOT : Serum Glutamic Oxaloacetic Transaminase, SGPT : Serum Glutamic Pyruvic Transaminase, Bilirubin : Serum Bilirubin, TC: Total Cholesterol, HDL Cholesterol and TGL: Triglyceride

Statistical analysis: ANOVA followed. The p-value is < .00001. The result is significant at  $p < .05$ .

There was no mortality in any of the groups. The body weight and relative liver weights of the experimental animals calculated at the end of the study had no statistically significant difference observed when compared to the control animals.

The mean concentration of Serum Biochemical investigations of Serum ACP; Serum Acid Phosphatase, ALP; Serum Alkaline Phosphatase, SGOT; Serum Glutamic Oxaloacetic Transaminase, SGPT; Serum Glutamic Pyruvic Transaminase, Bilirubin; Serum Bilirubin, TC; Total Cholesterol, HDL Cholesterol and TGL; Triglyceride

Body weight was measured weekly during the study period of 28 days wherein, no statistically significant changes were observed in the body weights of Test group animals as compared to Normal Control on respective days. Similarly, food consumption was also measured weekly wherein no statistically significant changes were observed in food consumption of Test group animals as compared to Normal Control.

The mean weight of liver was found in normal control rats is (3.854/g). With respect to experimental groups the minimum liver weight was recorded in rats treated with ETH and PnS

was (3.545/g) whereas maximum liver weight was recorded in rats treated with ETH, PAS and PnS (4.558/g) The body weight and relative liver weights of the experimental animals calculated at the end of the study had no statistically significant difference observed when compared to the control animals.

The mean concentration of Serum Acid Phosphatase was found in normal control rats was (0.06 IU/L). With respect to experimental groups the minimum concentration of Serum Acid Phosphatase found in rats treated with PnS was (0.04 IU / L), whereas maximum concentration of Serum Acid Phosphatase was found (0.07IU / L) in rats treated with ETH+PnS.

The mean concentration of Serum Alkaline Phosphatase was found in normal control rats was (287.67 IU / L). With respect to experimental groups the minimum concentration of Serum Alkaline Phosphatase found in rats treated with ETH and PnS was ( 286.67 IU / L), whereas maximum concentration of Serum Alkaline Phosphatase was found ( 540.14IU / L) in rats treated with ETH drug.

The mean concentration of Serum Glutamic Oxaloacetic Transaminase was found in normal control rats was (146.5 IU/L). With respect to experimental groups the minimum concentration of Serum Glutamic Oxaloacetic Transaminase

**Table 1.** Showing the mean concentration of Serum Biochemistry in *Sparague-Dawley* rats

Group	Wt. of liver/gm	ACP	ALP	SGOT (AST)	SGPT (ALT)	Bilirubin	Total Cholesterol	HDL Cholesterol	TGL
NC	3.854	0.06	287.67	146.5	44.87	0.29	89.33	24.43	144.83
PnS	4.121	0.04	331.5	154.63	52.53	0.33	94	25.63	143
ETH	3.949	0.06	540.14	149.43	84.27	0.39	92.57	21.2	183.43
PAS	3.853	0.06	349.38	155.75	85.82	0.36	89	11.38	157.38
ETH+PAS	4.075	0.06	480.75	166	50.59	0.32	86.5	14.96	134.25
ETH+PnS	3.545	0.07	286.67	160.33	52.01	0.27	66.33	16.53	134.83
PAS+PnS	4.129	0.05	324	181.43	54.65	0.25	63.71	18.17	115
ETH+PAS+PnS	4.558	0.05	371.5	165.5	56.08	0.29	89.17	20.73	132.83

\*Each value is the mean of 8 determinations.

ACP: Serum Acid Phosphatase IU / L

ALP : Serum Alkaline Phosphatase IU / L

SGOT : Serum Glutamic Oxaloacetic Transaminase IU / L

SGPT : Serum Glutamic Pyruvic Transaminase IU / L

Bilirubin : Serum Bilirubin mg / dl

TC: Total Cholesterol

HDL Cholesterol

TGL: Triglyceride

found in rats treated with PnS was ( 149.43 IU / L), whereas maximum concentration of Serum Glutamic Oxaloacetic Transaminase was found ( 181.43 IU / L) in rats treated with PAS+ PnS.

The mean concentration of Serum Glutamic Pyruvic Transaminase was found in normal control rats was (44.87IU/L ). With respect to experimental groups the minimum concentration of Serum Glutamic Pyruvic Transaminase found in rats treated with ETH+PAS was ( 50.59 IU / L), whereas maximum concentration of Serum Glutamic Pyruvic Transaminase was found ( 85.82IU / L ) in rats treated with PAS drug.

The mean concentration of Serum Bilirubin was found in normal control rats was (0.29 mg / dl). With respect to experimental groups the minimum concentration of Serum Bilirubin found in rats treated with PAS + PnS was (0.25 mg / dl ), whereas maximum concentration of Serum Bilirubin was found ( 0.39 mg / dl ) in rats treated with ETH drug.

The mean concentration of Total Cholesterol was found in normal control rats was (89.33mg / dl). With respect to experimental groups the minimum concentration of Total Cholesterol found in rats treated with ETH+ PAS was ( 63.71 mg / dl ), whereas maximum concentration of Total Cholesterol was found ( 94.00 mg / dl ) in rats treated with PnS.

The mean concentration of HDL Cholesterol was found in normal control rats was ( 24.43 mg / dl). With respect to experimental groups the minimum concentration of HDL Cholesterol found in rats treated with PAS was ( 11.38 mg / dl ), whereas maximum concentration of HDL Cholesterol was found (25.63 ) in rats treated with PnS.

The mean concentration of Triglycerides was found in normal control rats was (144.83 g / dl). With respect to experimental groups the minimum concentration of Triglycerides mg / dl found in rats treated with PAS and PnS was (115.00 g /dl), whereas maximum concentration of Triglyceride was found (183.43) in rats treated with ETH drug.

In our study, in case of ACP there was no statistically significant change observed in all groups when compared to normal control group. The levels of ALP were found to be significantly increased in ETH group ( $p < 0.001$ ) when compared to normal control animals. These levels were

found to be significantly decreased in ETH+PnS group ( $p < 0.001$ ) as well as ETH+PAS+PnS group ( $p < 0.01$ ). In PAS group, although the ALP levels were increased as compared to normal control animals the difference was not statistically significant. The ALP levels of ETH+PAS group were also found to be increased significantly ( $p < 0.01$ ) in comparison with normal control animals. Although these levels were decreased in ETH+PAS+PnS group, the difference was not statistically significant. In case of AST (SGOT) there was no statistically significant change observed in all groups when compared to normal control group. In case of ALT (SGPT) levels were significantly increased in ETH and PAS groups ( $p < 0.05$ ) as compared to normal animals. Although the ALT (SGPT) levels were found to be decreased in PnS treated groups, but the difference was not statistically significant. The bilirubin levels were found to be increased in PnS, ETH, PAS and ETH+PAS groups in comparison with normal control animals but the difference was not statistically significant. The levels were found to be significantly decreased in ETH+PnS group ( $p < 0.05$ ) when compared to ETH. Along with this, PAS + PnS group has also shown the significant decrease in bilirubin levels in comparison with PAS group ( $p < 0.05$ ). The cholesterol levels were found to be significantly decreased in PAS+PnS group in comparison with PAS group ( $p < 0.05$ ). The total Cholesterol levels were found to be significantly reduced in PAS group animals ( $p < 0.001$ ), ETH+PAS group ( $p < 0.001$ ) and in ETH + PnS ( $p < 0.05$ ) comparison with normal control. PAS has also significantly reduced HDL Cholesterol levels as compared to ETH group ( $p < 0.001$ ). In comparison with PAS, the HDL cholesterol levels were increased significantly in PAS + PnS ( $p < 0.05$ ) as well as ETH + PAS + PnS group ( $p < 0.01$ ), there was no statistically significant change observed in all groups when compared to normal control group.

fig. 1a and b: NC-Male & Female: the histological architecture of liver sections of healthy rats showed normal cellular architecture with distinct hepatic cells and sinusoidal space.

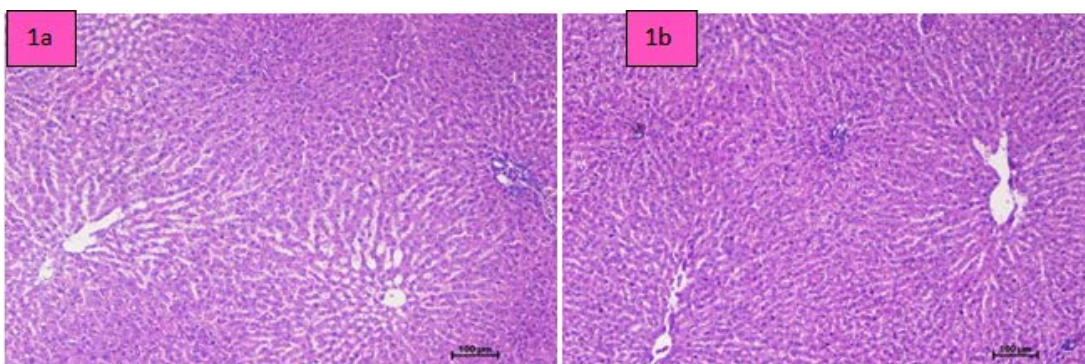
fig. 2a and b: PNS-Male & Female: the structural changes of the liver are normal and hepatocytes show no symptoms of necrosis and degeneration.



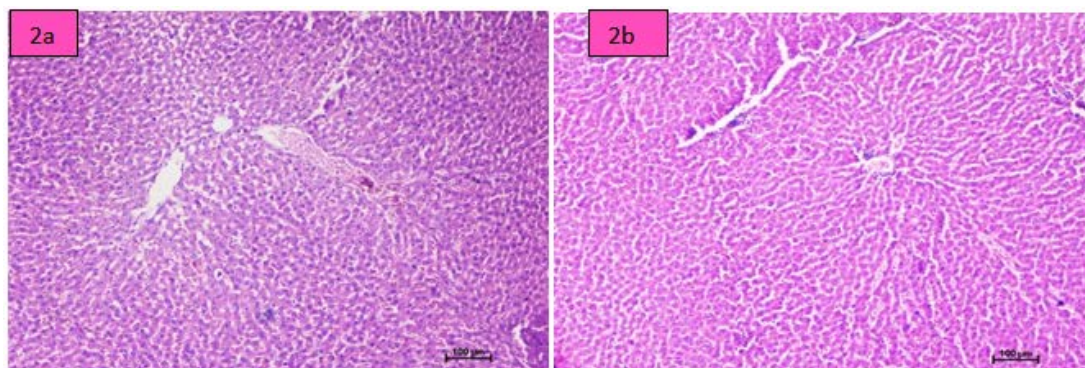
fig. 3a and b: ETH-Male & Female: the animals treated with ETH drug shows that mild degenerative effect of liver. In males congested vasculature in hepatic parenchyma. Focal cellular swelling and vacuolar changes of hepatocytes with degenerative changes were occasionally occurring. In females congested vasculature and focal minimal

degenerative changes in hepatic parenchyma. Multiple foci of cellular swelling and vacuolar changes in hepatocytes were seen with granular cytoplasm changes. Focal sinusoidal hemorrhages and focal MNC infiltration were occasionally seen.

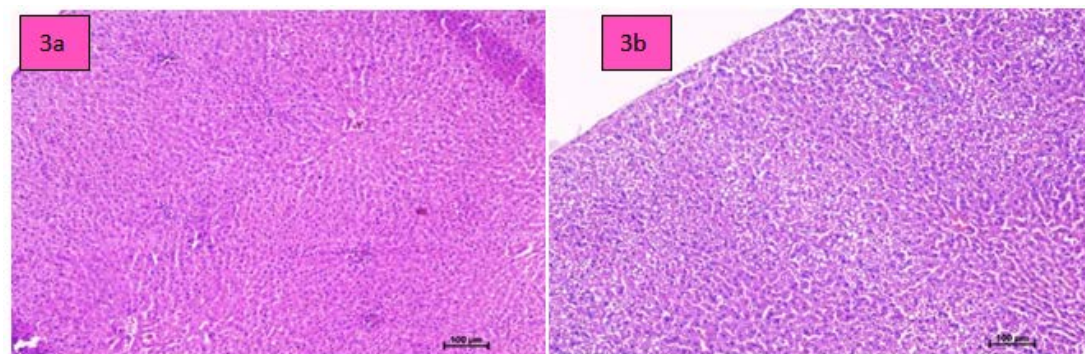
fig. 4a and b: PAS-Male & Female: the animals treated with PAS drug showed mild



**Fig. 1a & b.** NC-Male & Female: the histological architecture of liver sections of healthy rats showed normal cellular architecture with distinct hepatic cells and sinusoidal space



**Fig. 2a & b.** PNS-Male & Female: the structural changes of the liver are normal and hepatocytes show no symptoms of necrosis and degeneration



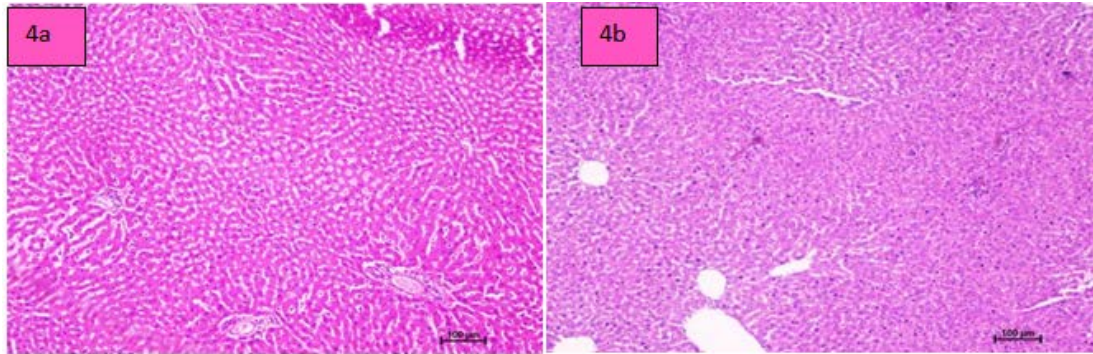
**Fig. 3a & b.** ETH-Male & Female: the animals treated with ETH drug shows that mild degenerative effect of liver



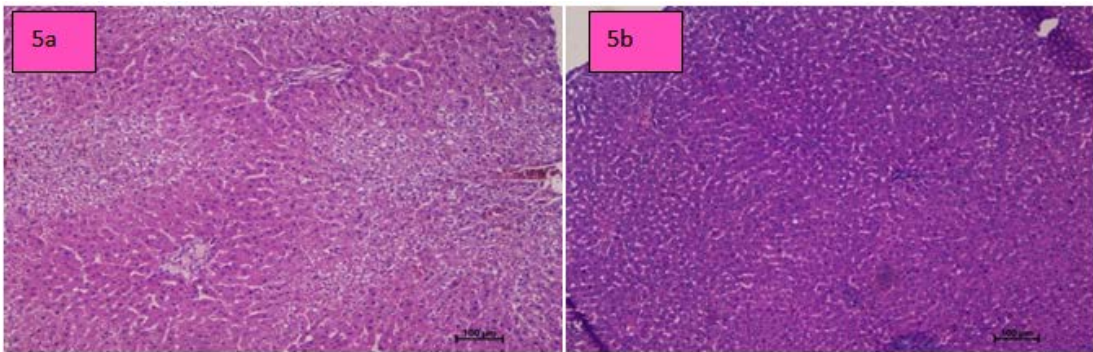
degenerative effect of liver. In males congested vasculature in hepatic parenchyma. Focal cellular swelling and vacuolar changes of hepatocytes with degenerative changes of hepatocytes occasionally observed. In females normal hepatic parenchyma with normal histomorphology of hepatocytes and portal vascular tissue were observed. The hepatocytes arranged in cord like manner around

central vein with presence of normal sized nucleus and intact cellular borders. Normal bile ducts and portal areas were seen.

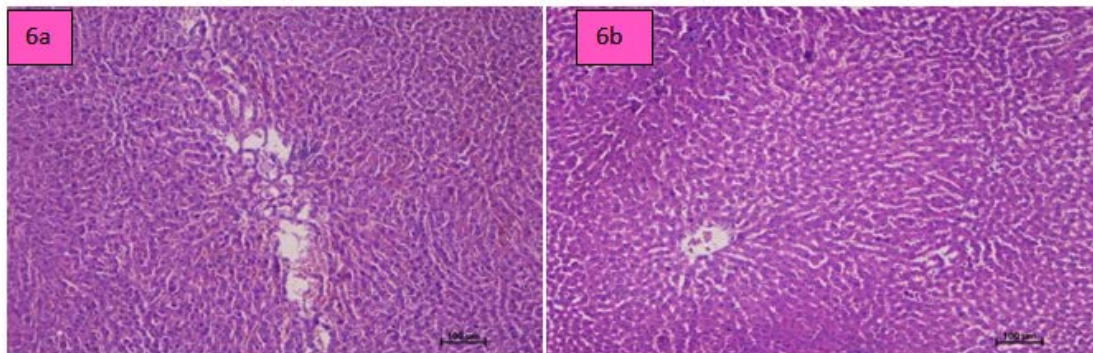
fig. 5a and b: ETH+PAS- Male & Female: in the liver section of the rats intoxicated with ETH+PAS drug, there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis. Congested vasculature and



**Fig. 4a & b.** PAS-Male & Female: the animals treated with PAS drug showed mild degenerative effect of liver



**Fig. 5a & b.** ETH+PAS- Male & Female: in the liver section of the rats intoxicated with ETH+PAS drug, there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis



**Fig. 6a & b.** ETH+PNS- Male & Female: in the liver section of the rats intoxicated with ETH drug in combination with PNS, there was rearrangement and regeneration of normal hepatic cells

focal minimal degenerative changes were occurring in hepatic parenchyma with cellular swelling and vacuolar changes of hepatocytes. Fatty infiltration in hepatocytes occurred. In female normal hepatic parenchyma with normal histomorphology of hepatocytes and portal vascular tissue were seen. The hepatocytes arranged in cord like manner around central vein with presence of normal sized nucleus and intact cellular borders. Normal bile ducts, and portal areas was clearly seen.

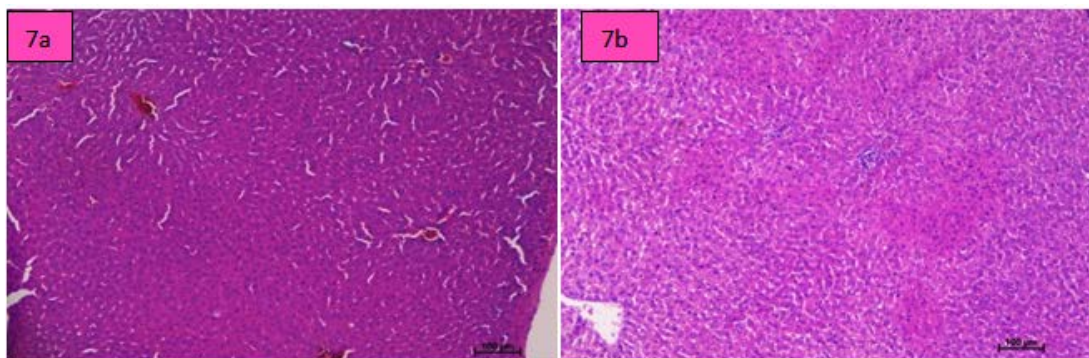
fig. 6a and b: ETH+PNS- Male & Female: in the liver section of the rats intoxicated with ETH drug in combination with PNS, there was rearrangement and regeneration of normal hepatic cells with normal hepatic parenchyma with normal histomorphology of hepatocytes and portal vascular tissue. The hepatocytes arranged in cord like manner around central vein with presence of normal sized nucleus and intact cellular borders.

fig. 7a and b: PAS+PNS- Male & Female: in the liver section of the rats intoxicated

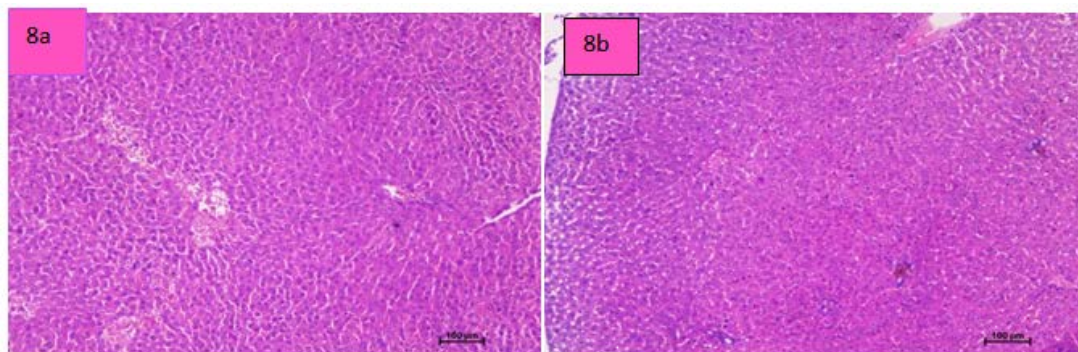
with PAS drug in combination with PNS, there was rearrangement and regeneration of normal hepatic cells with normal hepatic parenchyma with normal histomorphology of hepatocytes and portal vascular tissue. The hepatocytes arranged in cord like manner around central vein with presence of normal sized nucleus and intact cellular borders.

fig. 8a and b: ETH+PAS+PNS- Male & Female: in the liver section of the rats intoxicated with ETH+PAS drug in combination with PNS, there was rearrangement and generation of normal hepatic cells with normal hepatic parenchyma. histomorphology of hepatocytes and portal vascular tissue showed normal arrangement. The hepatocytes arranged in cord like manner around central vein with presence of normal sized nucleus and intact cellular borders.

Hepatotoxicity is one of the most frequent and serious adverse effects of anti-TB medications and may reduce treatment effectiveness by compromising treatment regimens<sup>23, 24</sup>. Different



**Fig. 7a & b.** PAS+PNS- Male & Female: in the liver section of the rats intoxicated with PAS drug in combination with PNS, there was rearrangement and regeneration of normal hepatic cells with normal hepatic parenchyma



**Fig. 8a & b.** ETH+PAS+PNS- Male & Female: in the liver section of the rats intoxicated with ETH+PAS drug in combination with PNS, there was rearrangement and generation of normal hepatic cells with normal hepatic parenchyma



experimental studies on animals suggest that administration of antitubercular drugs results in the rise of ALT, AST and ALP in serum, affecting hepatocellular membrane integrity and its organelles<sup>25, 26</sup>. Increased activity of hepatocytes leads to hyperbilirubinaemia which helps to determine integrity of liver<sup>27</sup>. It has been reported that sub acute or chronic treatment with isoniazid induced hepatotoxicity in man<sup>28</sup>, rat<sup>29</sup>, and guinea pigs<sup>30</sup> resulting in the rise of serum transaminases and phosphatase activities. Isoniazid-induced hepatitis is associated with ballooning degeneration, focal hepatocyte necrosis with minimal cholestasis<sup>31</sup>.

In our study, it was found that piper nigrum at the higher dose levels prevented an increased in ALT, AST, ALP, ACP, Bilirubin, levels.

The increased levels of AST and ALT are indicative of cellular damage and loss of functional integrity of the cell membrane in the liver<sup>32</sup>. The increase in ALP in liver disease is the result of increased synthesis of the enzyme by cells lining the canaliculi, usually either intra- or extra hepatic, which reflects the pathological alteration in biliary flow<sup>33</sup>. An abnormal increase in the levels of bilirubin in serum indicates hepatobiliary disease and severe disturbance of hepatocellular function<sup>34</sup>. The study carried out by<sup>35</sup> found that piperine inhibited the increased level of serum GPT and GOT in dose-dependent manner in a hepatotoxicity model of mice caused by D-galactosamine. The hepatoprotective activity of methanolic extract of Piper nigrum fruits was evaluated in ethanol-CCl<sub>4</sub> induced hepatic damage in Wistar rats. In their study they showed significant liver protection as evidenced from the triglycerides levels, Alanine transaminase, Aspartate transaminase, alkaline phosphatase, bilirubin and superoxide dismutase, Catalase, Glutathione reductase and Lipid peroxidation levels to assess the liver functions. The study carried out by<sup>36</sup> showed administration of Ethanol-CCl<sub>4</sub> exhibited significant boost in triglycerides, Alanine transaminase, Aspartate transaminase, alkaline phosphatase, and bilirubin levels while there was significant decrease in the superoxide dismutase, catalase, and glutathione reductase levels which were restored to normal level after pre-treatment of methanolic extract of Piper nigrum and Piperine. The Morphological

and histopathological studies of liver were also supportive of the biochemical parameters. Thus it is concluded that Piper nigrum possesses potential hepato-protective activity due to the presence of piperine alkaloids and have great therapeutic potential in treatment of liver ailments<sup>37</sup>. Piperine is one of the constituent of Piper nigrum is an active ingredient against acetaminophen induced hepatotoxicity studied by<sup>38</sup> in mice. It has hepatoprotective potential and known to be used as therapeutic medicine for various ailments<sup>39</sup>. The hepatoprotective effect of aqueous extract of Piper longum and piperine against first line antituberculous drugs having antioxidant property and hence proves its hepatoprotective potential<sup>40</sup>. Our study beholds the similar view. In our study we have found the intracellular hepatotoxic markers have increased in drugs treated animals and were controlled when treated with Piper nigrum in single or in combination of both the drugs.

## CONCLUSION

Administration of Ethionamide and Para amino salicylic acid in Sprague-Dawley rats for 28 days showed hepatotoxicity in test groups. Hepatotoxicity was confirmed by biochemical parameters with the support of histoarchitecture examination of liver. Ethanolic extract of Piper nigrum (Linn.) seeds were administered to the test groups have ameliorated the toxic effect of the drugs. Based on the above results it is concluded that the *Piper nigrum* (Linn.) acts as hepato-protective agent hepato-toxicity caused by Ethionamide and Para amino salicylic acid in Sprague-Dawley rats and in combination of both. *Piper nigrum* (Linn) showed the hepatoprotective activity against the drugs however the molecular studies are necessary to elucidate mechanism of action of hepatoprotective activity of *Piper nigrum* (Linn.) against Ethionamide and Para amino salicylic acid.

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