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Original Research Article

Ameliorative Effect of Piper Nigrum on Ethionamide and Para Amino Salicylic Acid Induced Nephrotoxicity in Sprague- Dawley Rats

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Abstract

Fresh seeds of *Piper nigrum* were procured from the botanical garden of Kokan Krushi Vidyapeeth, 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 either sex were used for the experiment. The ETH and PAS drugs 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 of Serum Albumin, Urea, Creatinine, Total proteins and Blood Urea Nitrogen (BUN). The kidney tissues were analyzed for Histopathology. Graph Pad Prism 7 was used for statistical analysis by one way variance (ANOVA). The value $p< 0.05$ considered as significant. Ethanolic seed extract of *Piper nigrum* (Linn.) was administered independently as well as in combination with ETH and PAS drugs. It is found that the pretreated test groups with *Piper nigrum* ameliorated the toxic effect of the drugs. *Piper nigrum* (.Linn) also showed the normalization of histoarchitecture of the kidney by confirming nephroprotective activity against ETH and PAS drugs. Based on the above results it is concluded that the *Piper nigrum* act as nephroprotective agent and a good bio-enhancer against nephrotoxicity induced by ETH and PAS drugs in Sprague-Dawley rats.

Keywords: Piper nigrum, drugs, nephrotoxicity, bioenhancer.

INTRODUCTION

Tuberculosis is an infection that affects around one-third of the world's population and leads to millions of deaths around the globe. It also accounts for the highest mortality and morbidity worldwide [1]. Drug-resistant TB is the global concern which estimates about 558000 people found to develop TB and were resistant to rifampicin and among those 82% have detected with MDR-TB (multidrug resistant tuberculosis). India holds first rank for (MDR-TB) as the acute kidney injury and chronic kidney diseases due to the use of rifampicin in tuberculosis treatment which is a serious concern today [2]. Second-line drugs are important in drug-resistant TB and their use is increased in recent years. The adverse side effects of these drugs raise the risk of renal impairment if not supervised critically.

Drug induced nephrotoxicity is common complication in certain patients and special clinical cases [3]. Nearly twenty percent of community and hospitalize patients are related acute kidney failure due to drug induced toxicity [4, 5]. Anti tuberculosis drugs like Amino glycosides and cyclic-polypeptides are well known for causing nephrotoxicity, ototoxicity, vestibular toxicity, electrolyte abnormalities and other rare side effects [6]. The TB patient has higher risk of chronic kidney disease (CKD) [7].

Ethionamide (ETH) is key drug used in MDR-TB drug resistance tuberculosis and have to shown physiological action [8-10]. It is listed as one of the essential medication in drug resistance tuberculosis in children and adults [11]. Para-amino salicylic acid (PAS) was the first antibiotic found to be efficient in the treatment of tuberculosis in the 1940s [12]. PAS enhances the activity of drug isoniazid and streptomycin and is extensively used in the combinations against Mycobacterium tuberculosis [13]. 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 [14].

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 [15]. Now days the dietary supplements and herbal remedies have increase the interest of researchers to treat different kind of diseases. In India, over 40

poly-herbal commercial formulations have hepatoprotective, nephroprotective and many others action is being used [16, 17].

Piper nigrum (Linn.) (PnS) (family Piperaceae) 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 [18]. Piper nigrum along with other phyto-constituents 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 [19]. In view of the above property of *Piper nigrum* the present study has been undertaken to find the effects of *Piper nigrum* on nephrotoxicity induced by Ethionamide and Para amino salicylic acid in Sprague-Dawley rats.

MATERIALS AND METHODS

a) Collection of Sample

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

b) 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^{0}C till further use. The weight of the powder was calculated based on weight of the seeds.

c) 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.

d) Experimental Design

Sixty four (64) *Sprague- dawley* rats (average weight 150 - 240 g) of either 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/Re Bi Rc /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^{0}C; humidity, 12-hlight/12 hrs dark cycle was also maintained by feeding the rats with commercial rat pallets and water available ad libitum.

Table-1: Effect of ethanol extract of Piper nigrum seeds and drugs Ethionamide Para amino salicylic acid, on Sprague- Dawley rats

Groups	Specification	Treatment specifications
1	(NC)	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

e) 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.

f) Biochemical assay

Test drug and inducers were given to respective groups as indicated in the table daily for 28 days. At the end of study animals were sacrificed by heart puncture to analyze the various biochemical parameters of the serum such as Albumin, Urea, Creatinine, Total proteins and Blood Urea Nitrogen (BUN) by standard methods.

g) Histological analysis:

The Kidney 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, as proposed by [20] and histological structure of kidney tissue were examined under the Biological digital microscope-Motic B1 Series.

Graph Pad Prism 7were used for statistically analysed by one way analysis of variance (ANOVA) The value $p< 0.05$ considered as significant.

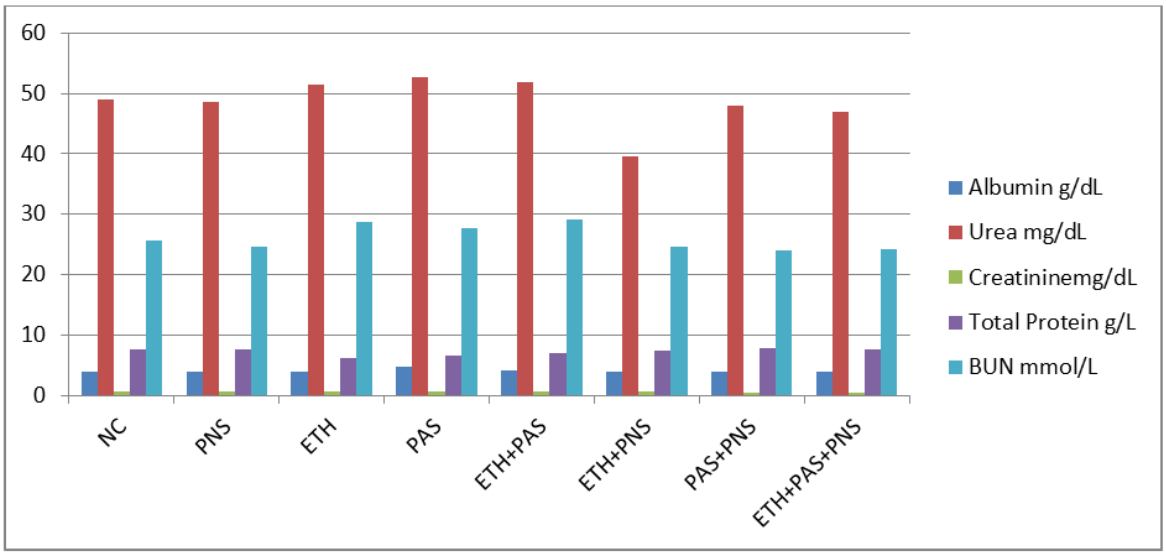
RESULTS AND DISCUSSIONS

I) Statistical Analysis

Table-2: Showing the mean concentration of Serum Biochemistry of Effect of ethanol extract of Piper nigrum seeds and drugs Ethionamide Para amino salicylic acid, on Sprague- Dawley rats

Group	Weight of kidney tissue /g	Albumin g/dL	Urea mg/dL	Creatinine mg/dL	Total Protein g/L	BUN mmol /L
NC	0.796	3.82	49.08	0.57	7.52	25.65
PNS	0.786	3.96	48.67	0.58	7.6	24.54
ETH	0.842	4.01	51.34	0.61	6.21	28.67
PAS	0.748	4.75	52.64	0.62	6.58	27.68
ETH+PAS	0.834	4.14	51.75	0.58	7.0	29.05
ETH+PNS	0.713	3.92	39.63	0.57	7.45	24.67
PAS+PNS	0.735	3.94	47.86	0.52	7.84	23.98
ETH+PAS+PNS	0.804	3.91	46.89	0.49	7.59	24.15

*Each value is the average of 8 determinations.



Graph-1: Showing the graphical representations of Serum Biochemistry Sprague- Dawley rats

Histological analysis of Kidney of Sprague-Dawley rats

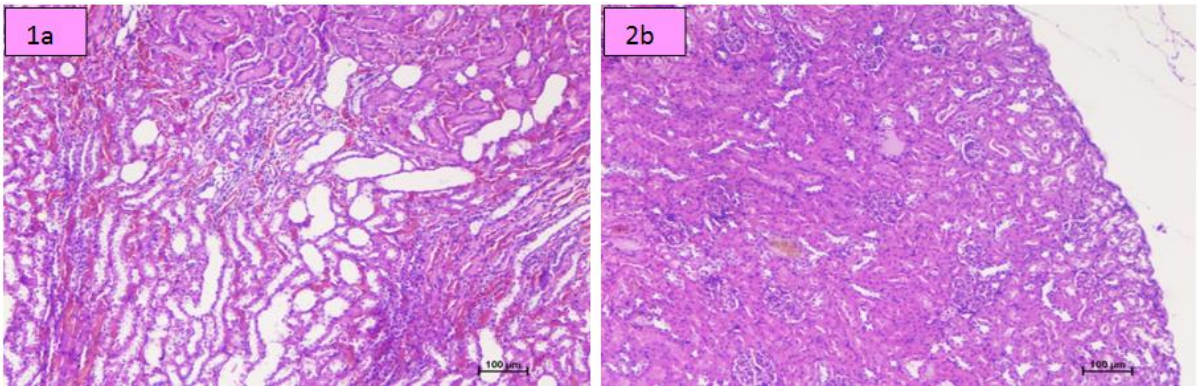


Fig 1a and b: NC Male and Female: The histological architecture of kidney sections of healthy rats showed normal histomorphology of glomeruli and renal tubules.

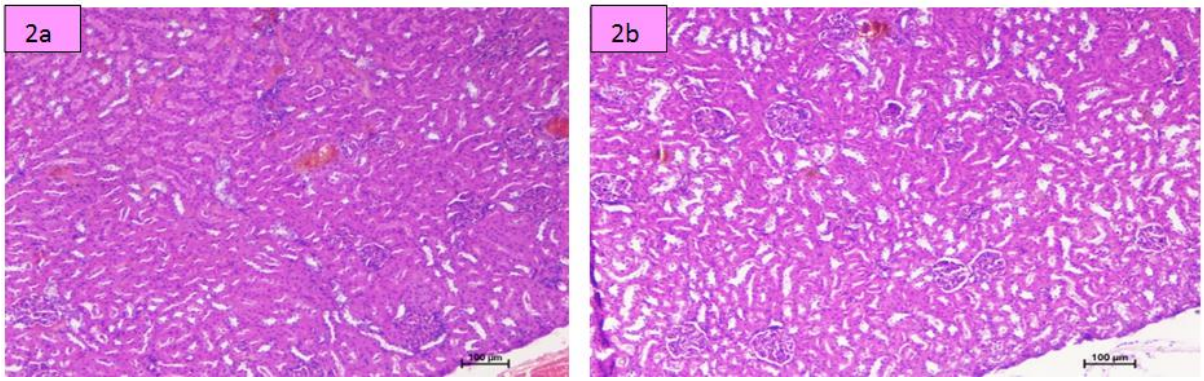


Fig 2a and b: PNS- Male and Female: The histological architecture of kidney sections of healthy rats showed normal histomorphology of glomeruli and renal tubules

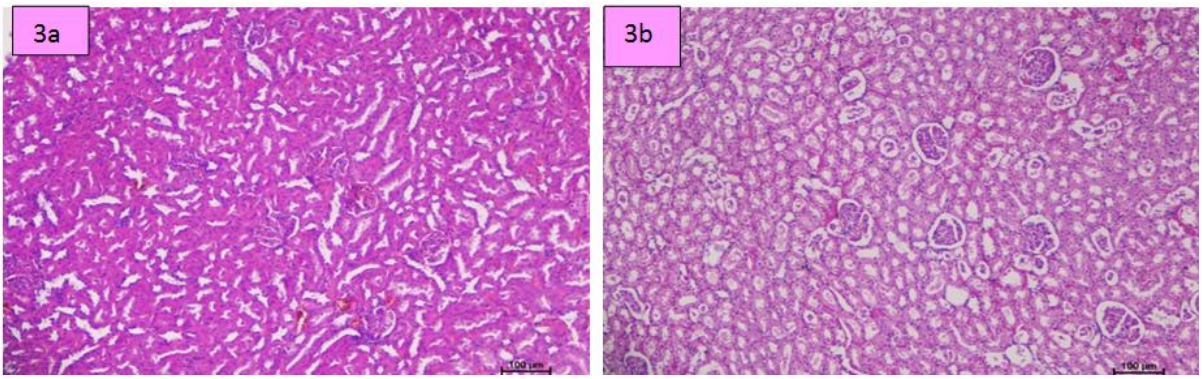


Fig 3a and b: ETH- Male and Female: Kidney showed focal Congestion of vessels in renal parenchyma

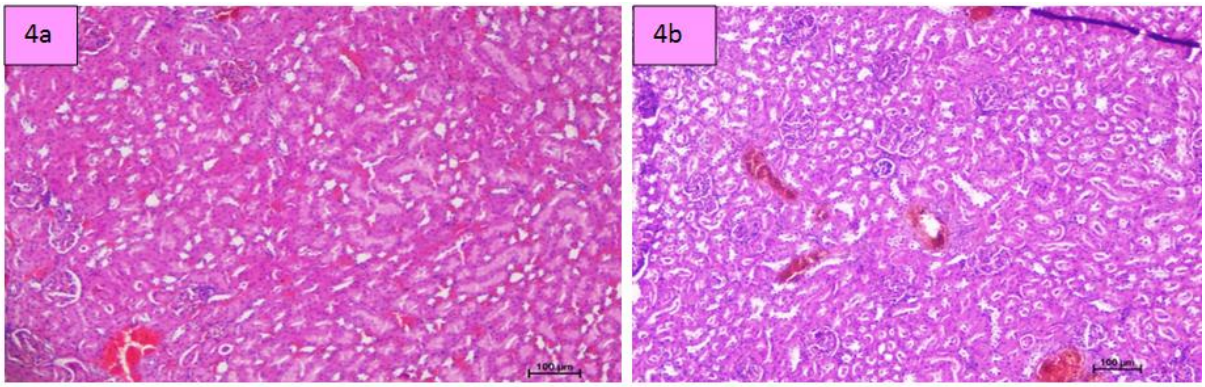


Fig 4a and b: PAS- Male and Female: Kidney showed focal Congestion of vessels in renal parenchyma, swelling of renal tubules with the presence of granular cytoplasmic changes in the epithelium of renal tubules

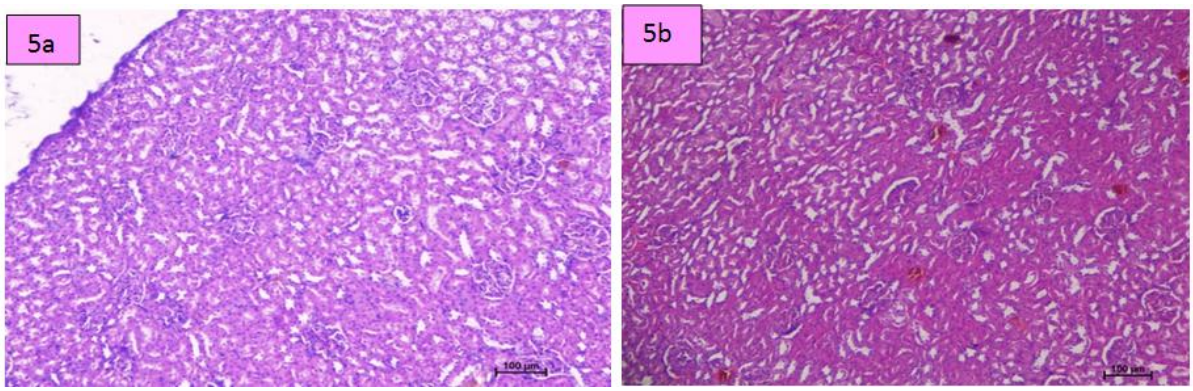


Fig 5a and b: ETH+ PAS- Male and Female: Congestion of vessels in cortex and medulla of renal parenchyma in females with degenerative foci of nephrosis and focal atrophic changes of glomeruli

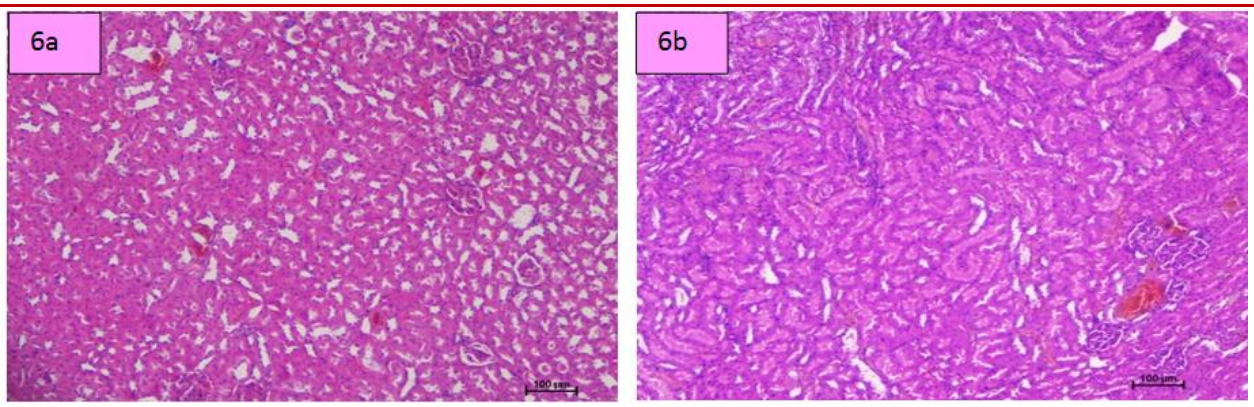


Fig 6a and b: ETH+ PNS- Male and Female: The presence of granular cytoplasmic changes in the epithelium of tubules in males and normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in tubules in females

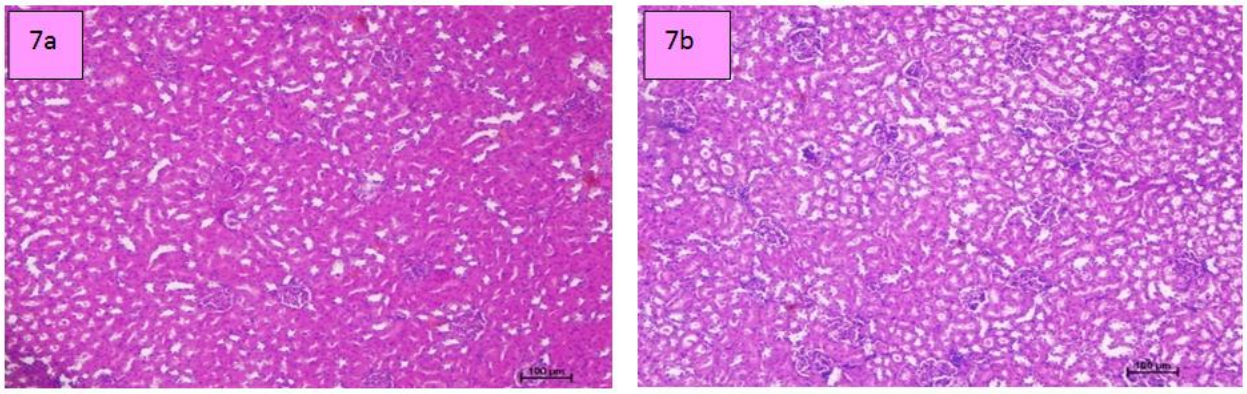


Fig 7a and b: PAS + PNS- Male and Female: The histological architecture of kidney sections showed normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in tubules

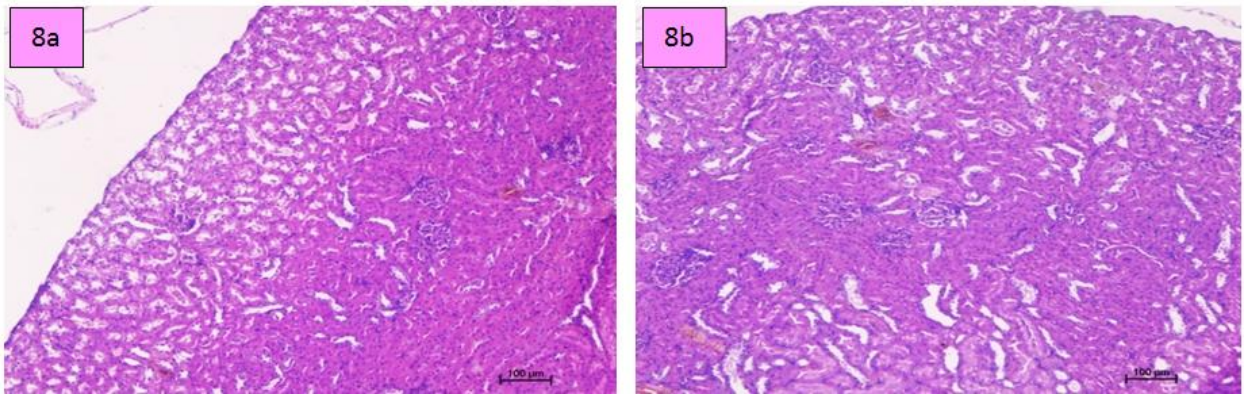


Fig 8a and b: ETH+PAS + PNS- Male and Female: The histological architecture of kidney sections showed normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in tubules

Table-2 & Graph-1 The mean concentration of serum biochemical investigations of serum Albumin, Urea, Creatinine, Total proteins and Blood Urea Nitrogen(BUN) have been investigated by using standard methods for the estimation of renal functional test. There was no mortality in any of the groups in Sprague- Dawley rats.

The mean weight of kidney was found in normal control rats is (0.796 /g). With respect to experimental groups the minimum mean kidney weight was recorded in rats treated with ETH and PnS was (0.713/g) whereas maximum mean kidney weight was recorded in rats treated with ETH (0.842/g) The body weight and relative kidney weights of the experimental animals calculated at the end of the study had no statistically significant difference when compared to the control animals.

The mean concentration of serum albumin was found in normal control rats was (3.82 g/dL). With respect to experimental groups the minimum mean

concentration of serum albumin found in rats treated with ETH , PAS , and PnS was (3.91 g/dL), whereas maximum mean concentration of serum albumin was found (4.75 g/dL) in rats treated with PAS drug.

The mean concentration of serum urea was found in normal control rats was (49.08 mg/dL). With respect to experimental groups the minimum mean concentration of serum urea found in rats treated with ETH and PnS was (39.63 mg/dL), whereas maximum mean concentration of serum urea was found (52.64 mg/dL) in rats treated with PAS drug.

The mean concentration of serum Creatinine was found in normal control rats was (0.57mg/dL). With respect to experimental groups the minimum mean concentration of serum Creatinine found in rats treated with ETH, PAS, and PnS was (0.49 mg/dL), whereas maximum mean concentration of serum Creatinine was found (0.62 mg/dL) in rats treated with PAS drug.

The mean concentration of serum Total Protein was found in normal control rats was (7.52 g/L). With respect to experimental groups the minimum mean concentration of serum Total Protein found in rats treated with ETH was (6.21g/L), whereas maximum mean concentration of serum Total Protein was found (7.84 g/L) in rats treated with PnS and PAS drug.

The mean concentration of serum Blood Urea Nitrogen (BUN) was found in normal control rats was (25.65 mmol /L). With respect to experimental groups the minimum mean concentration of serum Blood Urea Nitrogen (BUN) found in rats treated with PAS and PnS was (23.98 mmol/L), whereas maximum mean concentration of serum Blood Urea Nitrogen (BUN) was found (29.05 mmol/L) in rats treated with ETH and PAS drug.

Histological Analysis

Fig 1a and b to 8a and b: Microscopic examination of Kidney tissues showing alterations in different groups in Sprague-Dawley rats as:

Fig 1a and b: NC Male and Female: The histological architecture of kidney sections of healthy rats showed normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in tubules.

Fig 2a and b: PNS- Male and Female: The histological architecture of kidney sections of healthy rats showed normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in tubules.

Fig 3a and b: ETH- Male and Female: Focal congestion of vessels in renal parenchyma. The focal areas of cellular swelling of renal tubules with presence of granular cytoplasmic changes in the epithelium of tubules were seen.

Fig 4a and b: PAS- Male and Female: Normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in tubules in male and focal congestion of vessels in renal parenchyma. The renal tubules showed congested focal cellular swelling of with necrotic presence of granular cytoplasm with change in the epithelium of renal tubules.

Fig 5a and b: ETH+ PAS- Male and Female: Focal congestion of vessels in renal parenchyma. The renal tubules showed necrotic cellular swelling with presence of granular cytoplasmic changes in the epithelium in males and female. The congestion of vessels in cortex and medulla of renal parenchyma is observed in females with degenerative foci of necrosis and focal atrophic changes in glomerulus cells. The renal tubules showed presence of granular cytoplasm with changes in the tubules.

Fig 6a and b: ETH+ PNS- Male and Female: Focal congestion of vessels in renal parenchyma. The focal areas with mild cellular swelling of renal tubules with presence of granular cytoplasm in males and normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in tubules were seen in females.

Fig 7a and b: PAS + PNS- Male and Female: The histological architecture of kidney sections showed normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in renal tubules.

Fig 8a and b: ETH+PAS + PNS- Male and Female: The histological architecture of kidney showed normal histomorphology of glomeruli and renal tubules with intact cellular features and intact nucleus in renal tubules.

The ETH and PAS are major drugs used to treat the MDR-TB. nephrotoxicity due to these drugs has lesser concerned of the researcher around the globe and has not been seriously studied. A very scanty data is available in respect of second line drugs like ETH and PAS against nephrotoxicity. Even though hepatoprotective study of *Piper nigrum* have been extensively studied by the researcher, but very little attention has been paid to reveal the effect of *Piper nigrum* on nephrotoxicity induced by second line drugs like ETH and PAS.

The study carried out by [21] on *S. fusiformis* against toxicity induced by INH and RIF on rat. They showed that rat treated with INH and RIF resulted in increased levels of serum creatinine, urea and uric acid thereby indicating impaired renal function. They also

showed that the animals treated with *S. fusiformis* treatment showed reversal changes in histoarchitecture of kidney by lowering the levels serum creatinine, urea and uric acid with reversal changes in the antioxidant status of the kidney. The antioxidant status of the kidney was reversed due to *S.fusiformis.* In another study carried out by [22] reported lead acetate treatment induced significant elevation of serum creatinine and BUN activities. The lead acetate significantly increased creatinine and BUN levels, which can be an indicator of impaired renal function in nephrotoxicity. The serum creatinine and BUN are recommended for the assessment of kidney injury in preclinical studies as it is considered as a more specific and sensitive indicator of kidney damage. The study carried out by [23] showed low levels of serum, creatinine and BUN are normally found in the blood but when the kidney is damaged or diseased, creatinine and BUN levels increases. The study carried by [24] indicated that piperine has a nephroprotecctive activity against lead acetate induced nephrotoxicity, in their study they found that the pretreated groups with piperine shows an improvement in the creatinine and BUN levels as well as piperine showed regeneration in tubular epithelial cells. They have also examined the protective effect of piperine in lead acetate induced nephrotoxicity in rats and observed the improvement in kidney histopathology and decrease in the level of BUN (Blood urea nitrogen), creatinine and MDA (Malondialdehyde) due to lead acetate and significant increase in SOD (Super oxide dismutase) and GPx (glutathionine peroxidase) due to piperine. In the study carried by [25] stated that the Piper longum Linn decreases the lipid peroxidation in serum, liver, kidney and also insignificantly increases the level of GSH glutathione in tissue against monosodium glutamate (MSG) oxidative stress in rats. They concluded that the *Piper longum* Linn provide significant safety to liver and kindney from MSG induced oxidative stress.

In our study, our results are in agreement with the results carried out in above said earlier studies. In our study we found that the animals pretreated with ETH and PAS independently or in combination showed in increased levels of serum albumin, urea, creatinine, and blood urea nitrogen (BUN). It is also seen that the levels of serum total protein have decreased due to ETH and PAS which indicating their loss from the blood due to damaged renal function which beholds similarities with study carried by researchers in different studies. In our study creatinine levels were found to be non significantly altered in test groups in comparison with normal control animals except for ETH, PAS and PnS, where the levels were significantly decreased ($p<0.01$) which may lead to the impaired renal function. There was no significant changes were observed in the Albumin, Urea, and Total Protein. In our one of the antioxidant study we found that the levels of SOD, GSH, and catalase get decreased in rats pretreated with ETH and PAS independently or in combination. When the rats pretreated with *piper nigrum* independently or in combination with drug ETH and PAS showed an improvement in the levels of SOD, GSH, and catalase, this may be because of piperine which enhances the level of antioxidant enzymes in the serum. The ETH and PAS showed minimal to mild changes associated with histopathology of kidney in both male and female rats. In the said study we found the animals treated with *Piper nigrum* independently or in combination with the drug ETH and PAS altered the serum biochemistry by lowering the levels of albumin, creatinine, urea, BUN except total serum protein and also towards the normalization of histoarchitectural structure of the kidney. The above result suggests that the ethanolic extract of *Piper nigrum* have nephroprotective activity.

CONCLUSION

Administration of Ethionamide and Para amino salicylic acid in Sprague-Dawley rats for 28 days showed nephrotoxicity in test groups. Nephrotoxicity was confirmed by biochemical parameters with the support of histoarchitecture examination of kidney. Ethanolic extract of *Piper nigrum* (Linn.) seed extract was administered independently as well as in combination with ETH and PAS drugs. We found that the pretreated test groups with *Piper nigrum* showed ameliorated the toxic effect of the drugs. *Piper nigrum* (.Linn) showed the nephroprotective activity against the drugs. Based on the above results it is concluded that the *Piper nigrum* act as nephroprotective agent and a good bioenhancer against nephrotoxicity induced by ETH and PAS drugs in Sprague-Dawley rats.

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