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OXIDATIVE STRESS INDUCED BY ETHIONAMIDE AND PARA AMINO SALICYLIC ACID CAUSE AMELIORATION BY PIPER NIGRUM (LINN.) IN SPRAGUE- DAWLEY RATS.

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ABSTRACT The intention of this study is to explore the antioxidant potential of ethanolic seed extract of Piper nigrum (Linn.) (PnS) in Ethionamide (ETH) and Para amino salicylic acid (PAS) induced oxidative stress in rats. Rats, orally administered with ETH 132 mg/kg and Para amino salicylic acid 400 mg/kg ethanolic seed extract of PnS 500 mg/kg for 28 days. Control animals were fed with food pellets and water ad libitum. Assay of anti oxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) were determined by taking liver tissue homogenate. In our study we have found the intracellular hepatotoxic marker enzymes showed oxidative stress by decreasing antioxidant levels in drugs treated animals and were controlled when treated with *Piper nigrum* in single or in combination of both the drugs which confirms the oxidative stress induced by ETH and PAS cause amelioration by *Piper nigrum* in Sprague- Dawley rats.

KEYWORDS : ETH, PAS, CAT, SOD, GSH.

INTRODUCTION

Liver is one of the largest and vital organs of human body and is vulnerable for tissue insult continuously. Liver regulates various important metabolic functions, the distortion of which causes hepatic damage [1] .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More than 900 drugs have been implicated in causing liver injury [2] and it is the most common reason for a drug to be withdrawn from the market.

Para-amino salicylic acid (PAS) was the first antibiotic found to be efficient in the treatment of tuberculosis in the 1940s [3]. 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 [4]. PAS may cause the hepatitis [5]. These two drugs are causes various adverse reactions in different organs at a time. It is mandatory to shade a light on the oxidative damage done by theses drugs due to scanty information with curative measure.

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 [6]. *Piper nigrum* (Linn.) (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 [7]. *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 [8]. 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 cause the adverse reactions leading to cellular damage in the human beings. Therefore, in the present study, attention has been given to find the effect of *Piper nigrum* on oxidative stress induced by ETH and PAS in the Sprague-Dawley rats.

MATERIALS AND METHODS

Collection of sample:

Fresh *Piper nigrum* seeds 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.

Extraction:

The ethanolic extract of the *Piper nigrum* 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.

Purchase 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 four (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/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 240C; humidity, 12-hlight/12 hrs dark cycle was also maintained by feeding the rats with commercial rat pallets and water available ad libitum.

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.

Groups	Specification	Treatment specifications
1	Normal control	Rate pellets and water ad libitum
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

Biochemical assay

At the end of the study animals were anesthetized and were sacrificed by cervical decapitation. Liver tissues were excised and studied for CAT, SOD, GSH by standard methods.

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:

Table: 1. Showing the mean concentration of antioxidants in Sprague-Dawley rats

Group	CAT Catalase (mM/min/gm of tissue)	SOD(U/gm of wet tissue)	GSH (mg/gm of wet tissue)
NC	292.01	431.351	6.3
PnS	152.18	250.225	6.75
ETH	155.05	255.341	4.394
PAS	151.42	262.387	4.14
ETH+PAS	120.43	261.261	4.325
ETH+PnS	218.16	361.562	7.267
PAS+PnS	262.2	347.748	9.954
ETH+PAS+PnS	301.15	386.787	9.513

*Each value is the mean of 8 determinations. CAT: Catalase ; SOD: Superoxide dismutase ; GSH: Glutathione

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.

In our study it was found the levels of liver catalase were reduced significantly in ETH ($p<0.01$), PAS ($p<0.01$) and ETH+PAS ($p<0.001$) groups compared to normal control group. It was also observed that treatment with PnS elevated reduced levels of catalse significantly in PAS+PnS group ($p<0.05$). Similarly levels of catalase were upregulated significantly in ETH + PAS + Pns group compared to its respective diseases control group ($p<0.001$).

Levels of SOD were also reduced in disease groups but change was not significant. These levels were elevated significantly in ETH+PnS ($p<0.001$), PAS + PnS ($p<0.001$) and ETH + PAS + Pns ($p<0.001$) in comparison with respective disease control group.

Further levels of GSH were reduced in disease control (ETH, PAS, ETH+PAS) but change was not statistically significant. These depleted levels were elevated in treatment groups ETH+PnS ($p<0.01$), PAS + PnS ($p<0.001$) and ETH + PAS + PnS ($p<0.001$) in comparison with respective disease control group.

Various diseases and medical conditions invite the cellular damage in various organs which can be ameliorated by use of herbs [9]. Superoxide and hydroxyl ions are highly toxic at higher concentrations produced during the stress, in medicinal treatments or in unhealthy environmental conditions. SOD; CAT and GSH are the enzyme which scavenges these harmful molecules and protect the cell from tissue injury. SOD and CAT levels can be maintained on the treatment with spices like, pepper, ginger, garlic as they control the lipid peroxidation during cell injury [10] . GSH detoxifies the oxygen species harmful to the cells and thus provide the support to minimize the tissue damage. In the study carried by [11],it is concluded that piperine positively affects the SOD, Glutathione Per Oxidase GPx , BUN, Creatinine levels in lead acetate induced nephrotoxicity in rats. In the study carried by[12] it is seen that Isoniazid induces the mitochondrial dysfunction, decrease in GSH level and histoarchitectural displacements in hepatoma cell line (Hep-G2) indicating the hepatotoxicity. The study carried out by [13], found that piperine inhibited the increased level of serum GPT and GOT in dose-dependent manner in a hepato-toxicity model of mice caused by D-galactosamine. The study carried out by[14], on The hepatoprotective activity of methanolic extract of Piper nigrum fruits was evaluated in ethanol- CCl4 induced hepatic damage in Wistar rats , 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[15] showed administration of Ethanol-CCl4 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 hepatoprotective effect of aqueous extract of Piper longum and piperine against first line antituberculous drugs having antioxidant property and hence proves its hepatoprotective potential[16]. Our study beholds the similar view. In our study we have found the intracellular hepatotoxic marker enzymes showed oxidative stress by decreasing antioxidant levels in drugs treated animals and were controlled when treated with Piper nigrum in single or in combination of both the drugs which confirms the oxidative stress induced by ETH and PAS cause amelioration by Piper nigrum in Sprague- Dawley rats.

CONCLUSION:

Administration of Ethionamide and Para amino salicylic acid in Sprague-Dawley rats for 28 days showed oxidative stress in test groups by suppressing the level of antioxidant enzymes as CAT, SOD and GSH. Whereas after administration of Ethanolic extract of PnS to the test groups in combination with the drugs as well as independently showed an increased levels of enzymes CAT, SOD and GSH. Based on the above results it is concluded that PnS have the potential against oxidative stress induced by ETH and PAS in Sprague-Dawley rats.

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Conflict of Interest:

There is no conflict of interest in the present work.

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