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# Medicinal Impact of Piper- Nigrum (Piperine) Against Arsenic Induced Hepatic and Renal Toxicity in Experimental Mice.

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## ABSTRACT

**Background:** In view of the increasing risk of arsenic on human health, the present study has been carried out to investigate the hepato-protective effect of piperine on arsenic induced-hepatic and renal toxicity in mice. Various oxidative stress parameter, antioxidant level and micro nutrients were analyses in hepatic and hepatic renal organ of mice. **Methods:** Mice exposed arsenic (sodium arsenate 5 mg/kg body weight p.o. for 45 days) caused a significant increases oxidative stress in hepatic and renal tissue as compared to controls group. **Results:** Abnormal levels of arsenic in hepatic and renal tissue increased the levels of ROS, LPO, and decreased the levels of GSH with SOD, CAT, and GPx activities in the hepatic and renal tissue of mice as compared to controls. Co-treatment of arsenic with piperine (1.5 mg/kg body weight p.o. for 45 days) decreased the levels of ROS, LPO, and increased the level of GSH, also increased SOD, CAT, and GPx activity and showed improvements in hepatic and renal tissue of mice as compared to arsenic-treated groups. **Conclusion:** Our results proved that piperine worked as antioxidant, anti- inflammatory in nature.

***Keywords:*** Hepatic toxicity, renal toxicity, Piperine.

## INTRODUCTION

Millions of people around the globe are exposed to unsafe levels of arsenic due to consumption of contaminated drinking water. Its sub-toxic levels may not be fatal, but the accumulation of lower levels of arsenic for a longer period of time leads to chronic exposure and cause adverse health effects, including metabolic disorders [Pace et al., 2018; Spratlen et al.,2018]. The toxic effect of arsenic has been found to be increased in malnourished population as they are mainly depends on the available water contaminated with arsenic [Zablotska et al. 2008]. Both the USEPA and the World Health Organization have adopted drinking water standard of 10µg/L (10ppb) [WHO and USEPA, 2017].

High levels of arsenic has been reported in three districts Ballia, Varansi and Gazipur of Uttar Pradesh in the upper and middle Ganga plain, India [Ahamed et al.,2006]. The soluble salts of arsenic including arsenate or arsenite are well absorbed (80%) through the gastrointestinal tract and cause health effects in individuals. Further, individuals suffers from arsenicosis have high risk to develop other health related disorders including cardiovascular, hepatic, renal, gastrointestinal, neurological and reproductive problems and malignancies [Brinkel et al.,2009; Kapaj et al.,2006].

Due to the accumulative properties of arsenic, deposition of high concentrations of arsenic in the liver, kidney, lungs, hair and nails have been well reported as a result of chronic exposure [Klaassen., 1996]. In view of increasing risk of chronic arsenic toxicity, the World Health Organization has lowered the permissible limit of arsenic in drinking water from 50 µg/L to 10 µg/L [WHO., 2001].

The metabolic function of the liver is primarily responsible for detoxification of toxins and carcinogens. Drug induced liver injury may manifest as acute hepatitis, cholestasis, and further develop as liver cirrhosis. Reactive oxygen species (ROS) generated by metabolic intermediates of xenobiotics via induction of CYP450 families as well as activated inflammatory cells through NADPH oxidases promote the accumulation of lipid derived oxidation products that cause liver injury, resulting in cell necrosis [Liu et al.,2000]. Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its

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unique metabolic functions and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics.

Besides, industrial applications have also contributed to disperse arsenic in the environment. High levels of arsenic in ground water in India and many other regions of the world have been associated with various health problems including arsenicosis, skin lesions, cardiovascular diseases, reproductive problems, psychological, neurological and immunotoxic response (Duker et al., 2005; Kapaj et al., 2006; Brinkle et al., 2009; Flora et al., 2011).

Kidney is the major excretory and osmoregulatory organ that plays an important role in the control and regulation of homeostasis with reabsorption, secretion and metabolic functions [Goswami et al., 2005]. Arsenic is capable of causing acute as well as chronic renal damage. It has been reported previously that many heavy metals and metalloids (e.g. Lead, mercury, cadmium and so on) are nephrotoxic [Sharma et al., 2007; Tiwari et al., 2004].

Recent research have shown that the plant derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness and proved to be safe, better patient tolerance, relatively less expensive plant extracts and phyto-constituents with free-radical scavenging properties could have great importance as therapeutic agents in several diseases associated with enhanced oxidative stress [Khan., 2009; Flora., 2011]

Piperine, main component of Piper nigrum, is a plant alkaloid with a long history of medicinal use in Indian medicine. It has been extensively used as a condiment and flavoring for all types of savory dishes to enhance the taste and flavour of food since ancient times [Platel and Srinivasan, 2004]. It is known to exhibit a variety of biological and physiological activities [Srinivasan 2007] including antidepressant [Li et al, 2007], anti-metastatic [Pradeep and Kuttan 2002] antiapoptotic [Choi et al 2007], antioxidant [Pathak and Khandelwal 2008], immunomodulatory and antitumor [Sunila and Kuttan 2004; Pathak and Khandelwal 2008]. Piperine also had an inhibitory effect on lung metastases probably due to NF-kß inhibition and pro inflammatory cytokine gene expression. in fact, piperine possessed only weak cytotoxic activity [Sunila and Kuttan 2004, Bezerra et al., 2005], indicating that its antitumor activity is not related to direct antiproliferative effects on tumor cells. Piperine has been reported to inhibit in vitro and in vivo production of nitric oxide and tumor necrosis factor-a and to inhibit lung metastasis .Vijayakumar et al., 2004 reported that simultaneous supplementation with piperine in rats fed high fat diet lowered thiobarbituric acid reactive substances (TBARS) and conjugated dienes levels and maintained superoxide dismutase, catalase, GPX, glutathione-S- transferase (GST) and glutathione levels close to controls in rats. Selvendiran et al., [2005 a,b] observed that supplementation of piperine caused inhibition of phase I and II enzymes, elevation of glutathione metabolizing enzymes, reduction in DNA damage and DNA protein cross-links in benzo (a) pyrene induced lung carcinogenesis in mice. Studies are also reported to show the protective effect of piperine against benzo (a) pyrene induced DNA damage, DNA- protein cross links and lung carcinogenesis in swiss albino mice, [Selvendiran et al., 2005 a, b.] Abo-Zeid [2009] reported the antigenotoxic and antimutagenic activity of piperine that may be useful for reducing and preventing the DNA damages induced by carcinogens in somatic and germ cells.

## MATERIALS AND METHODS

### Animals and Treatment

The Balb/c male mice (15 ± 2 g) were obtained from the animal breeding colony of CSIR-Indian Institute of Toxicology Research, Lucknow used for the study. Mice were housed in an air-conditioned room at 25 ± 2°C with a 12 h light/dark cycle under standard hygiene conditions and had free access to pellet diet and water ad libitum. The study was approved by the institutional animal ethics committee of King George Medical University, Lucknow (No. 121 IAH/Pharma-11) and all experiments were carried out in accordance with the guidelines laid down by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment and Forests (Government of India), New Delhi, India. The dose of arsenic and other herbal agents are selected on the basis of earlier studies available in the literature [Savabieasfahani et al., 1998; Institoris et al., 2002; Demerdesh et al., 2009]. People have carried out their studies from low to high dose of arsenic to show its hepatotoxic effects. The dose of piperine is based on the studies carried out by [Li et al., 2007; Abo-Zeid et al., 2009]d for the present study. The animals were randomly divided into four groups contained ten animals in each group as follows

Group I - animals were treated with vehicle for 45days (distilled water) and served as control.

Group II - animal were treated with piperine (1.5 mg/kg/ body weight p.o., dissolved in distilled water) 45 days.

Group III -animals were treated with arsenic (5.0 mg/kg/ body weight p.o., dissolved in distilled water) 45 days.

Group IV - animals were simultaneously treated with arsenic piperine for in group II and group III.

At the end of the 45 days, all surviving animals were sacrificed by cervical dislocation. Immediately after

sacrificing, the liver and kidney of each mice was removed, cleaned, weighed and stored at – 80°C until further analysis for enzymatic and non-enzymatic antioxidants assays.

**Estimation of intracellular ROS production-**

To measure intracellular ROS production according to Balasubramanyam et al (2003). cells after treatment was loaded with 10 µM DCFH-DA for 45 min. ROS levels was measured using spectro fluorimeter (Waters, USA 474 Scanning Fluorescence Detector, with an excitation set at 485 nm and emission at 530 nm) as a change in fluorescence because of the conversion of non-fluorescent DCFH-DA to the highly fluorescent compound 2', 7'-dichlorofluorescein (DCF) in the cells.

**Estimation of lipid per oxidation-**

Lipid per oxidation was analyzed by the method of Ohkawa et al (1979). The reaction mixture in a final volume of 3.0 ml contained the cell lysate, 100 µl of 10% SDS, 600 µl of 20% glacial acetic acid, 600 µl of 0.8% TBA, and water. The mixture was placed in a boiling water bath for 1 h and immediately shifted to crushed ice bath for 10 min. The mixture was centrifuged at 2500 × g for 10 min. The amount of thiobarbituric acid reactive substances (TBARS) formed was assayed by measuring the optical density of the supernatant at 535 nm against a blank devoid of the cell lysate. The activity was expressed as nmoles of TBARS/mg of protein using 1,1,3,3,-tetramethoxypropane (TMP) as standard.

**Estimation of antioxidant enzymes:**

Catalase— Catalase was assayed by the method of Aebi (1984).The cell supernatant was treated with ethanol (10 µl/ml) and were kept on ice for 30 min. Triton X-100 (1%) was added subsequently and kept on ice for 30 min. Supernatant was added to assay mixture which contained 0.5 M sodium phosphate buffer (pH 7.0) and 10 mM $H_2O_2$. The decrease in absorbance was measured at 240 nm. The activity was calculated using extinction coefficient 0.04 mmole−1cm−1. One unit of catalase activity is defined as the amount of enzyme required to decompose 1 mole of $H_2O_2$/min.

Super oxide dismutase (SOD)—SOD was assayed by the method of Marklund and Marklund(1974) with slight modifications. The assay is based on the ability of enzyme to inhibit auto-oxidation of pyrogallol. The cytosolic supernatant treated with triton X-100 (1%) was kept at 4°C for 30 min and was added to the assay mixture, which contained 0.05 M sodium phosphate buffer (pH 8.0), 0.1 mM EDTA and 0.27 mM pyrogallol. Solution of pyrogallol was made fresh in 10 mM HCl. The absorbance was measured for 5 min at 420 nm. One unit of SOD activity is defined as the amount of SOD required to cause unit change in absorbance per minute.

Glutathione peroxidese (GPx) — The activity of GPx was measured by the procedure described by Paglia and Valentine (1967).The procedure is an indirect measurement of GPx activity. GSSG (glutathione disulphide i.e. oxidized GSH) produced as a result of action of GPx was immediately reduced in the presence of excess GR there by maintaining a Constant level of GSH in the reaction system. The assay made use of oxidation of NADPH by GR, which could be measured at 340 nm. The final concentration in 3 ml reaction volume contained 50 mM sodium phosphate buffer (pH 7) containing EDTA (0.1 M buffer with 1 mM EDTA), 0.24 U/ ml yeast GR, 0.3 mM GSH, 0.2 mM NADPH, 1.5 mM $H_2O_2$ and cytosolic sample. Reaction was started by addition of NADPH and the decrease in absorbance was monitored at 340 nm for 5 min. The GPx activity was expressed as n moles of NADPH consumed /min/mg of protein.

**Metal estimation-**

The liver and kidney tissue were acid digestion procedure for measuring As, Ca, Cu and Zn in liver and kidney were carried out using conc.$HNO_3$ and $HClO_4$ (6:1) under low heat to complete carbonization. Known volumes of deionized water was added and filtered. The metal was analyzed on Perkin Elmer ASS. Respective standards were also used.

**Statistical analysis:**

The statistical analysis was carried out by Graph Pad Prism 3.02 using one way analysis of variance followed by Newman–Keuls test for multiple pairwise comparisons among the groups. All values have been expressed as mean ± SEM. P value $<0.05$ has been considered significant.

## RESULTS

Effect on the generation of reactive oxygen species in hepatic and renal tissue of mice: Effect of arsenic and its co-treatment with piperine on the generation of reactive oxygen species liver and kidney has been presented in [Figure 1]. Mice exposed to arsenic exhibited a significant increases in the generation of reactive oxygen species in liver (35%, $p<0.001$) and kidney (24%, $p<0.01$) as compared to controls. Co-treatment of arsenic with piperine decreases in the generation of reactive oxygen species in liver (17%, $p<0.01$) and kidney (18%, $p<0.05$) respectively as compared to those treated with arsenic alone. No significant effect on generation of reactive oxygen species was observed in mice treated with piperine alone as compared to controls [Figure 1].

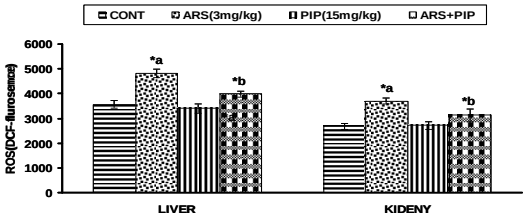


**Figure 1: Effect of arsenic, piperine and their co-treatment on the levels of reactive oxygen species in liver and kidney of mice**

Values are mean ±SEM of five animals in each group
*a-compared to control group, *b-compared to arsenic treated group
*Significantly differs ($p < 0.05$)

**Effect on the lipid per oxidation activity in hepatic and renal tissue of mice:** Effect of arsenic and its co-treatment with piperine on the lipid per oxidation level in liver and kidney has been presented in [Figure 2]. Exposure of arsenic to mice showed an increased lipid per oxidation in liver (39%, $p<0.001$) and kidney (37%, $p<0.01$) as compared to controls. Co-treatment of arsenic with piperine decreased the lipid per oxidation level in liver (26%, $p<0.01$) and kidney (15%, $p<0.05$) respectively as compared to mice treated with arsenic alone. No significant effect on the lipid per oxidation level was observed in mice treated with piperine alone as compared to controls [Figure 2].

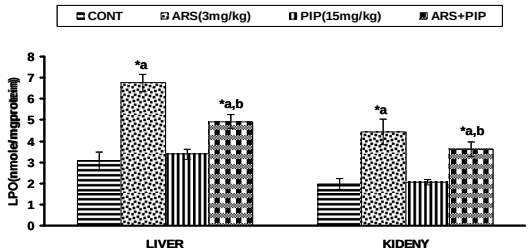


**Figure 2: Effect of arsenic, piperine and their co-treatment on the levels of lipid per oxidation in liver and kidney of mice**

Values are mean ±SEM of five animals in each group
*a-compared to control group, *b-compared to arsenic treated group
*Significantly differs ($p < 0.05$)

**Effect on the Catalase in hepatic and renal tissue of mice:** Arsenic has been found to be associated with the decreases in catalase. Effect of arsenic and co-treatment with piperine on Catalase reduced in liver and kidney has been presented in [Figure 3]. Exposure of arsenic to mice caused a reduced catalase level in liver (32%, $p<0.05$) and kidney (26%, $p<0.001$) as compared to controls. Co-treatment with arsenic and piperine increases the Catalase in liver (29%, $p<0.05$) and kidney (16%, $p<0.05$) respectively as compared to mice treated with arsenic alone suggested the antioxidant and free radical scavenging activity of piperine. No significant effect on the reduced Catalase was observed in mice treated and piperine alone as compared to controls [Figure 3].

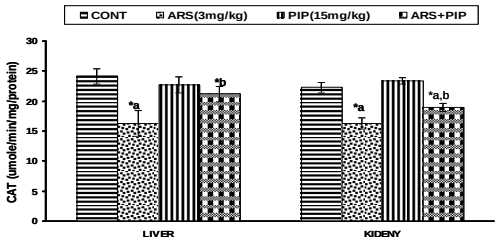


**Figure 3: Effect of arsenic, piperine and their co-treatment on the levels of catalase in liver and kidney of mice**

Values are mean ±SEM of five animals in each group
*a-compared to control group, *b-compared to arsenic treated group
*Significantly differs ($p < 0.05$)

**Effect on Super oxide dismutase of hepatic and renal tissue of mice:** Effect of arsenic and its co-treatment with piperine on super oxide dismutase in liver and kidney has been presented in [Figure 4]. Exposure of arsenic to mice showed decrease super oxide dismutase in liver (47%, $p<0.001$) and kidney (42%, $p<0.01$) as compared to controls. Co-treatment of arsenic with piperine increased the super oxide dismutase in liver (25%, $p<0.05$) and kidney (50%, $p<0.05$) respectively as compared to mice treated with arsenic alone. No significant effect on the super oxide dismutase in liver and kidney was observed in mice treated with piperine alone as compared to controls [Figure 4].

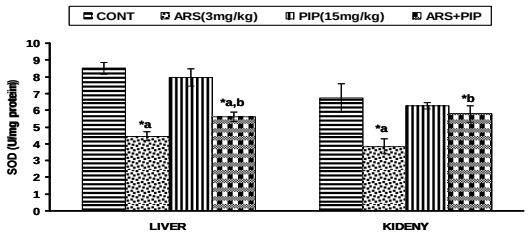


**Figure 4: Effect of arsenic, piperine and their co-treatment on the levels of super oxide dismutase in liver and kidney of mice**

Values are mean ±SEM of five animals in each group
*a-compared to control group, *b-compared to arsenic treated group
*Significantly differs ($p < 0.05$)

**Effect on the Glutathione peroxidese (GPx) in hepatic and renal tissue of mice:** Arsenic has been found to be associated with the decreases glutathione peroxidase. Effect of arsenic and co-treatment with piperine on Gpx level in liver and kidney has been presented in [Figure 5]. Exposure of arsenic to mice caused decrease Gpx in liver (43%, $p<0.01$) and kidney (44%, $p<0.01$) as compared to controls. Co-treatment with arsenic, and piperine increases the level in liver (54%, $p<0.05$) and kidney no significantly protection as compared to mice treated with arsenic alone suggested the antioxidant and free radical scavenging activity of piperine. No significant effect on the reduction of Gpx was observed in mice treated piperine alone as compared to controls [Figure 5].

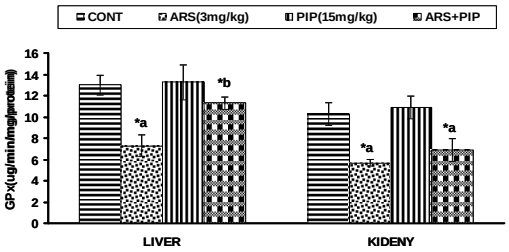


**Figure: 5. Effect of arsenic, piperine and their co-treatment on the levels of reduced glutathione peroxidase in liver and kidney of mice**

Values are mean ±SEM of five animals in each group
*a-compared to control group, *b-compared to arsenic treated group
*Significantly differs ($p < 0.05$)

**Effect of arsenic, piperine and co-treatment of arsenic with piperine on the Metal estimation in hepatic and renal tissue of mice:** Arsenic has been found to be associated with the decreases micro nutrient Ca, Zn level in hepatic and renal tissue and no significant level in Cu has been presented in [Table 1]. Exposure of arsenic to mice caused increase level of hepatic and renal tissue as compared to controls. Co-treatment with arsenic and piperine significantly increases the level Ca, Zn level in both tissue and also decreases in arsenic level in liver and kidney respectively as compared to mice treated with arsenic alone suggested the antioxidant and free radical scavenging activity of piperine. No significant effect was observed in mice treated piperine alone as compared to controls [Table 1].

**Table 1: Effect on, micro nutrient level in hepatic and renal tissue of mice exposed to arsenic, piperine and co-treatment of arsenic with piperine for 45 days**

| | Liver | | | | Kidney | | | |
|---|---|---|---|---|---|---|---|---|
| | As | Zn | Ca | Cu | As | Zn | Ca | Cu |
| CONT | 0.054+0.09 | 5.46±0.27 | 25.8±1.72 | 22.77±1.11 | 0.05±0.019 | 4.49±0.54 | 22.20±0.85 | 19.03±1.69 |
| PIP | 0.024±.003 | 5.83±0.04 | 33.37±1.24 | 22.2±1.18 | 0.05±0.019 | 5.70±0.34 | 23.47±0.57 | 19.27±0.56 |
| ARS | 2.33±0.20*a | 3.19±0.13*a | 20.67±0.84*a | 25.87±1.47 | 7.20±0.86*a | 2.51±0.21*a | 16.47±1.04*a | 20.90±0.72 |
| ARS+PIP | 1.44±.23*a,b | 4.25±0.17*b | 29.1±1.38*b | 23.23±0.54 | 3.28 5±0.37*a,b | 3.83±0.48*b | 20.47±1.16*b | 21.97±0.69 |

Values are mean ±SEM of five animals in each group
*a- compared to control group; *b- compared to arsenic treated group
*Significantly differs ($p < 0.05$)

## DISCUSSION

Piperine, naturally occurring spice component have good potential as antioxidant and hence utilized in nutritional and therapeutically preparations [Naidu, Thippeswamy., 2002]. Piperine is used as co-adjuvant for both treating as well as preventing the aging process and its related conditions like atherosclerosis, hypertension, diabetes, stress, depression, menopausal syndromes and benign prostate hypertrophy [Pistolesim.,2002]. Other study reported that against diabetes induced oxidative stress can be protected with piperine treatment for 14 days using diabetes mellitus as a model of oxidative damage [Rauscher et al., 2000]. In another study also proves the anti- inflammatory action of piperine comparable with curcumin derived from Curcuma longa. Along with anti-inflammatory activity piperine also shows antiarthritic activity [Bang et al., 2009].

Liver is a versatile organ of the body that regulates the internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its unique metabolic functions and related to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics. Arsenic primarily increased the generation of free radical species and cause an imbalance between pro-oxidation and antioxidant homeostasis in physiological system and cause toxicity due to its attraction towards the sulfhydryl groups of protein and thiols of glutathione. Thus an agent able to reduce the toxic potential of arsenic in liver cell would clearly be a use full compound for arsenical chemotherapy.

The liver has long been identified as a target organ of arsenic exposure [Santra et al., 2000], its importance as an organ arsenic biotransformation is well established [Rossman., 2003]. The mechanism by which arsenic directly damages the liver including oxidative stress [Das et al., 2005], enhanced inflammation and alteration in cellular methylation status has been investigated [Mazumder et al., 2009].

In vivo Studies are reported that, Mice exposed to arsenic also develop glomerular sclerosis, tubular necrosis, and increased oxidative stress kidney tissue [Liu et al., 2000; Li et al., 2010]. In vitro studies suggest that arsenic increases inflammation and oxidative stress [Escudero et al., 2010; Ned et al., 2010], and induces endothelial dysfunction [Shai et al., 2006].

## CONCLUSION

The findings of the present study clearly revealed that arsenic exposures in altered hepatic and renal oxidative stress, inflammation, decreased antioxidant level. It also altered the hepatic and renal mico-nutrients associated with enhanced oxidative stress in mice. Simultaneous treatment of arsenic and active constituent of piperine scavenge the arsenic induced free radicals and showed its anti-oxidant properties as evidence by balance the hepatic and renal micro nutrients in mice. The hepto and renal protective, of piperine could be responsible for its arsenic induced hepato and renal toxicity. Further

studies are required to understand the molecular mechanisms of arsenic induced oxidative stress and its protection by piperine.

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