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Original Article

Protective Role of Eugenol on Acute Promyelocyte Leukemia Drug Arsenic Trioxide Induced Renal Injury in Wistar Rats

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Abstract

Background: Arsenic trioxide (As_2O_3) has been shown to have substantial efficacy in treating refractory or relapsed acute promyelocytic leukemia (APL). However, its therapeutic potential was limited due to its toxic side effects in vital organs. The kidneys are a major elimination pathway for many antineoplastic drugs and their metabolites. Renal impairment can result in delayed drug excretion and metabolism of chemotherapeutic agents, resulting in increased systemic toxicity.

Objective: This study was designed to evaluate modulation of anti leukemic drug As_2O_3 induced renal toxicity by eugenol, a natural monoterpene found in clove oil.

Methods: Arsenic trioxide (4 mg/kg body weight) was given orally to Wistar rats for a period of 30 days. Renal function parameters (Urea, creatinine, creatinine clearance), enzymatic (Glutathione-S- transferase, glutathione peroxidase, superoxide dismutase, catalase) and non-enzymatic antioxidant (reduced glutathione), lipid peroxidation marker were analyzed. The kidneys were examined histopathologically for the confirmation of oxidative stress based injury in renal tissue.

Results: Oral administration of arsenic trioxide significantly increased renal function markers, lipid peroxidation byproduct level, and altered antioxidant system. Rats treated with arsenic trioxide had significantly higher oxidative stress and kidney arsenic accumulation. The co-treatment with eugenol (5 mg/kg body weight) significantly reduced the oxidative damage compare with arsenic treated group.

Conclusion: Our observations indicate that supplementation with monoterpenoid eugenol alleviated nephrotoxicity by improving antioxidant capacity in renal tissue. These findings suggest that eugenol act as a potential agent in combating arsenic trioxide induced renal toxicity.

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Introduction

Arsenic trioxide (As_2O_3) was discovered more than 2000 years ago for treating diseases such as syphilis, tuberculosis, cancer and in 2000, US Food and Drug

Administration approved As_2O_3 as a drug for the treatment of relapsed or refractory acute promyelocytic leukemia (APL) (Antman, 2001). But it has been associated with profound toxicity in the heart (Raghu and Cherian, 2009; Mathews et al., 2013; Mathews et al., 2016), liver (Mathews et al., 2012) and kidney (Zhang et al., 2001). Das et al (2005) reported that generation of reactive oxygen species (ROS) is very common in arsenic toxicity. Arsenic induces oxidative stress, which causes antioxidant defense system dysfunction and lead to oxidative damage to cellular macromolecules by increasing the generation of free radicals (Emadi and Gore 2010; Wang et al., 2012). The kidney is highly vulnerable to damage caused by ROS which was observed by Rodrigo et al (2002). Pharmacological and dietary strategies have targeted to control oxidative stress produced by ROS. Research findings have suggested that the administration of various antioxidants can prevent or subdue side effects of various chemotherapeutic agents. Although many naturally occurring molecules with excellent antioxidant properties are good candidates for prevention or control oxidative stress based tissue damage. Eugenol (1-allyl-4-hydroxy-3-methoxybenzene) has emerged as a potential food constituent with antioxidant potential (Kamatou et al., 2012). It is a naturally occurring phenolic compound from clove oil. Eugenol belongs to the class of essential oils that are generally recognized as safe (GRAS) by the Food and Drug Administration. On this background, the present study was under taken to explore whether eugenol can reduce the antileukemia drug As_2O_3 induces renal toxicity.

Materials and Methods

Chemicals and Reagents

Arsenic trioxide, Sodium pyruvate, Reduced glutathione (GSH), Oxidized glutathione (GSSG), Phenazine methosulphate (PMS), Nitroblue tetrazolium (NBT) were obtained from Sigma-Aldrich, Banglore, India. Eugenol was purchased from Hi Media Laboratories Pvt. Ltd, Mumbai. 2,4-dinitro bis Nitro benzoic acid (DTMB), Nicotinamide adenine dinucleotide (NADH), Thiobarbituric acid (TBA), Nicotinamide adenine dinucleotide phosphate (NADPH), 1-chlor, 2,4 dinitro benzene (CDNB), Potassium chloride (KCl), Ethylene diamine tetra acetic acid (EDTA), Hydrogen peroxide (H_2O_2), Trichloroacetic acid (TCA), Magnesium sulfate (MgSO4), Adenosine triphosphate (ATP), Ammonium molybdate, 8-anilino-1-naphthalesulfonic acid (ANSA), sodium nitrite, Orthophosphoric acid, Naphthyl ethylene diamine dichloride were purchased from Merk Specialities Pvt. Ltd, Mumbai, India. Other chemicals and solvents of analytical grade were purchased from the local retailer.

Animals and Treatment

Rats weighing 180-250 g were purchased from Pharmacology Unit, Nagarjuna Herbal Concentrates Ltd, Thodupuzha, Idukki, Kerala, India and acclimatized for six days. All the animals were maintained under standard laboratory conditions of temperature (25°C) and 12 hour light and dark cycles throughout the experiment period. The rats were provided with laboratory chow (Hindustan Lever Ltd. India) and tap water ad libitum. Experiments were conducted as per the guidelines of Institutional Animal Ethical committee, School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India (B29122014/3).

After an acclimatization period of one week, a total of 24 male rats were randomly allocated into four equally sized groups. Each group contains six rats; includes a normal control group, a eugenol control which received 5 mg/kg body weight of eugenol, a group administered with 4 mg/kg body weight of arsenic trioxide and a combination group treated with 4 mg/kg body weight of arsenic trioxide and 5 mg/kg body weight of eugenol.

At the end of the experimental period, all rats were sacrificed, blood was collected and serum was separated by centrifugation (3000 × g for 10 min). The kidneys were dissected out and washed in ice cold saline. The tissue was minced and homogenized accordingly with the experimental procedures.

Detection of Arsenic deposition in kidney

Kidney tissue was digested by thermal acid microwave digestion and diluted with double distilled

water. Total arsenic deposition in heart tissue was analyzed (Mathews et al., 2013) by standard inductively coupled plasma-optical emission spectroscopy (Optima 2000 DV ICP-OES, Perkin Elmer, Inc., Waltham, Massachusetts, USA).

Assay of tissue GSH

GSH was measured in tissue homogenate according to the method described by Ellman (1959). In the assay mixture contained 0.1 mL of sample, 0.85 mL of PBS (0.3 M, pH 7.4), and 0.05 mL of DTNB (10 mM). The reaction was read at 412 nm, and results were expressed as µmoles of GSH/g protein.

Assay of tissue GST

GST level was assayed by the method of Habig et al (1974). Tissue was washed in 1.15% KCl and homogenized in phosphate buffer (pH=7.4), centrifuged at 9000 rpm for 20 minutes. After centrifugation, the supernatant was mixed with 3 ml of the reaction mixture (1.7 mL Phosphate buffer + 0.1 mL of CDNB + 1.2 mL GSH) and it was measured spectrophotometrically at 340 nm.

Assay of Glutathione Peroxidase (GPx)

The activity of GPx was determined by the method of Rotruck et al. (1973). Briefly, the reaction mixture contained 0.2 mL of 0.4 M of Tris-HCl buffer (pH 7.0), 0.1 mL of 10 mM of sodium azide, 0.2 mL of homogenate (homogenized in 0.4 M of Tris-HCl buffer; pH 7.0), 0.2 mL of glutathione, and 0.1 mL of 0.2 mM of H_2O_2. The tubes were incubated at 37°C for 3 minutes, and the reaction was terminated by the addition of 0.5 mL of 10% trichloroacetic acid (TCA). To determine the residual glutathione content, the supernatant was removed after centrifugation and to this 1 mL of DTNB reagent was added. The color that developed was read at 412 nm against a reagent blank, and results were expressed as µg of GSH consumed/mg protein.

Assay of Superoxide dismutase (SOD)

SOD activity was determined by the method of Kakkar et al. (1984). The assay mixture contained 0.1 mL of sample, 1.2 mL of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 mL of PMS (186 µM), 0.3 mL of NBT (300 µM), and 0.2 mL of NADH (750 µM). The reaction was started by the addition of NADH. After incubation at 30°C for 90 seconds, the reaction was stopped by the addition of 0.1 mL of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 mL of n-butanol. The mixture was allowed to stand for 10 minutes, centrifuged, and the butanol layer was separated. The color intensity of the chromogen in butanol layer was measured at 560 nm against n-butanol, and the concentration of SOD was expressed as units/g of renal tissue. One unit was taken as the amount of enzyme that gave 50% inhibition of NBT reduction/mg protein.

Assay of Catalase (CAT)

Catalase activity in the sample was measured according to the method of Aebi (1974) by measuring the decrease in absorbance of H_2O_2 at 240 nm.

Assay of tissue TBARS

Thiobarbituric acid reactive substances (TBARS) activity was estimated by the method of Beuge and Aust (1978). That was measured after the addition of 2 mL of TBARS (15% w/v trichloroacetic acid and 0.25 N of HCl) to l mL of heart tissue homogenate, then heated in a boiling water bath for 15 minutes. After cooling, the suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was then separated, and absorbance was measured at 535 nm. The results were expressed as nM of TBARS formed/mg protein.

Blood urea, serum creatinine, serum γ – glutamyl transferase (GGT), creatinine clearance test were detected (Agappe Diagnostic Ltd., Ernakulam, Kerala, India) using UV-Visible Spectrophotometer (U-5100, Hitachi High Technologies, America, Inc.).

Histopathology

Small sections of kidney were fixed in 10% buffered formalin and processed for embedding in paraffin. Sections of 5-6 µm were stained with hematoxylin and eosin and examined for histopathological changes

under the microscope (Motic AE 21, Wetzlar, Germany). The microphotographs were taken using Moticam-1000 camera at the original magnification of 100X.

Statistical Analysis

The experimental results were expressed as mean±SD. Statistical analysis was evaluated by one-way analysis of variance (ANOVA) using SPSS software (version 20.0, SPSS Inc, Chicago, IL, USA).Values were considered significantly different if P<0.05.

Results

Effect of eugenol on arsenic accumulation in the renal tissue is shown in Fig. 1. Co-treatment with eugenol attenuated arsenic accumulation in the kidney compared with arsenic trioxide treated group. A significant (p<0.05) increase in the level of blood urea nitrogen, urea and creatinine were observed in arsenic treated rats when compared with normal rats. Administration of eugenol (5 mg/kg body weight) along with arsenic trioxide (4 mg/ kg body weight) significantly (p<0.05) restored the levels of urea, uric acid and creatinine near to normal levels when compared with arsenic alone treated rats. Eugenol did not alter the level of BUN, uric acid, and creatinine. Rats treated with arsenic showed a high level of γ-glutamyl transferase activity with a decrease (p<0.05) rate of creatinine clearance compared to control. Eugenol treatment provided marked protective effect and improvement on these parameters (Table I).

TABLE I: Effect of eugenol on renal function diagnostic markers in rats.

Parameters	*Normal control*	*Arsenic trioxide*	*Eugenol*	*Arsenic trioxide+Eugenol*
Blood Urea Nitrogen (BUN) (mg/dl)	20.02±1.21	78.94±1.49a	18.49±1.02	40.95 ±0.94b
Serum Urea (mg/dl)	32.45±0.21	102.69±0.28a	31.09±0.13	64.85 ±0.37b
Serum Creatinine (mg/dl)	0.98±0.04	4.16±0.06a	0.85±0.02	1.29 ±0.04b
Creatinine Clearance (ml/min)	0.69±0.03	0.17±0.02a	0.61±0.05	0.48 ±0.01b
γ-glutamyl transferase activity(U/L)	100.02±1.48	523.11±1.93a	94.27±1.86	248.08 ±2.14b

Normal control, Arsenic trioxide (4 mg/kg body weight), Eugenol (5 mg/kg body weight), Arsenic trioxide (4 mg/kg body weight) + Eugenol (5 mg/kg body weight). Data represented as mean ± SD, n=6. 'a' (significant difference between Normal control and Arsenic trioxide), 'b' (significant difference between Arsenic trioxide and Arsenic trioxide +Eugenol). P< 0.05.

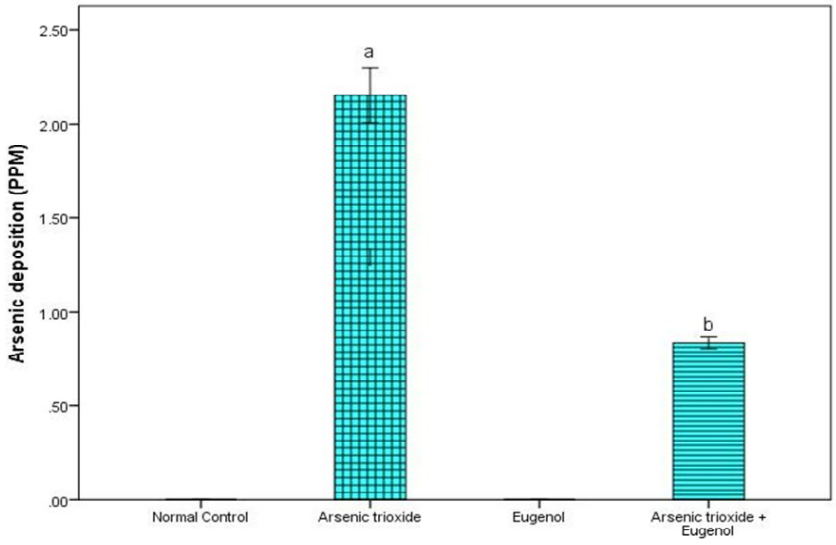

Fig. 1: Effect of eugenol on arsenic deposition pattern in the kidney. Experiment consist of Normal control, Arsenic trioxide – 4 mg/ kg body weight, Eugenol – 5 mg/kg body weight, and Arsenic trioxide – 4 mg/kg body weight) + Eugenol – 5 mg/ kg body weight. Data represented as mean±SD, n = 6. 'a' (significant difference between Normal control and Arsenic trioxide), 'b' (significant difference between Arsenic trioxide and Arsenic trioxide + Eugenol). P<0.05.

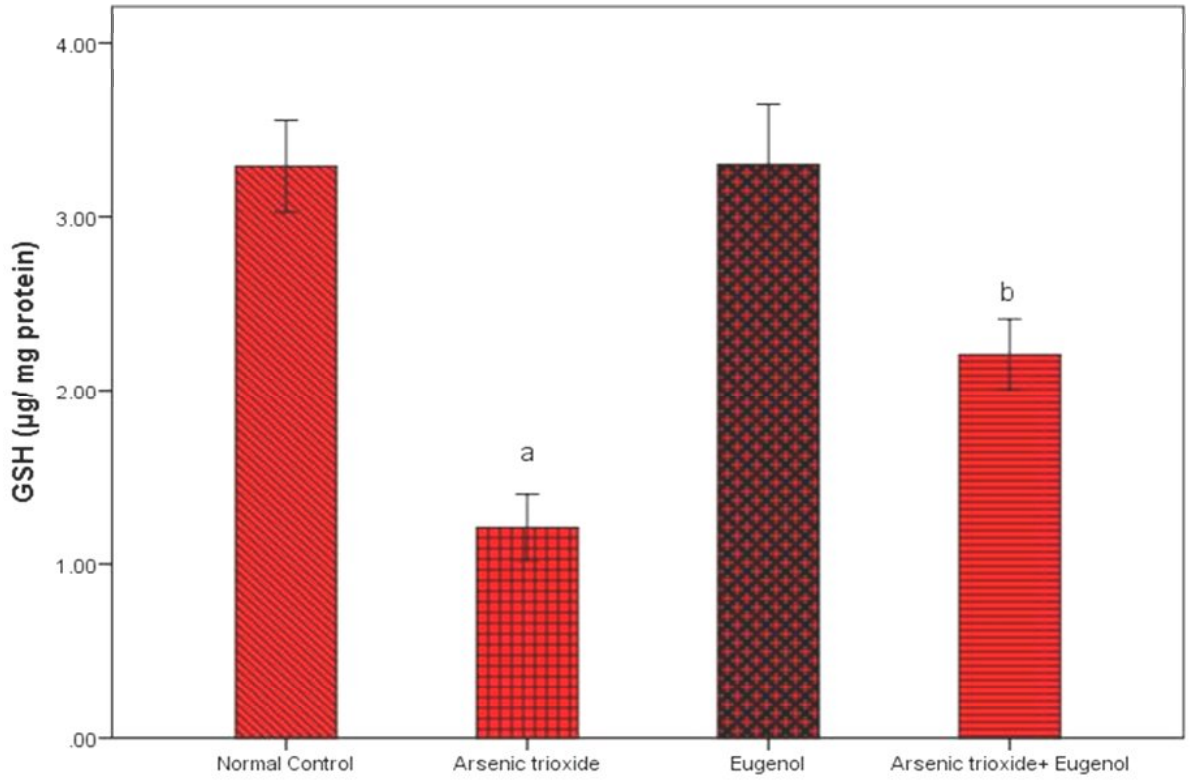

Fig. 2 : Effect of eugenol GSH in kidney. Experiment consist of Normal control, Arsenic trioxide – 4 mg/kg body weight, Eugenol- 5 mg/kg body weight, and Arsenic trioxide – 4 mg/ kg body weight) + Eugenol – 5 mg/kg body weight. Data represented as mean±SD, n=6. 'a' (significant difference between Normal control and Arsenic trioxide), 'b' (significant difference between Arsenic trioxide and Arsenic trioxide + Eugenol). P<0.05.

Treatment of normal rats with eugenol produced significant changes in kidney GSH level (Fig. 2) compared to normal controls. Treatment of rats with arsenic significantly ($p<0.05$) decreased the activity of GSH. Administration of eugenol (5 mg/kg body weight) along with arsenic trioxide significantly ($p<0.05$) increased the level of non-enzymatic antioxidant reduced glutathione in renal tissue. The changes in the activities of enzymatic antioxidants GST, GPx, SOD and CAT in control and experimental rats were shown in Fig. 3, 4, 5 and Fig. 6 respectively. Significant decreases ($p<0.05$) in the activities of these enzymes were observed in arsenic treated rats. However, treatment with eugenol caused a significant ($p<0.05$) increase in GST, GPx, SOD & CAT activity compared with arsenic treated group. These findings suggest that eugenol exerted its protective effects on arsenic induced renal toxicity may via the antioxidant pathway. The arsenic treated rats showed significant ($p<0.05$) elevated renal tissue TBARS level compared to controls (Fig. 7). Co-treatment of rats with eugenol attenuated the arsenic trioxide increases in MDA level in kidney.

Histopathological change is a direct indication of renal injury. The H&E-stained renal tissues appeared to have normal kidney tubules in the control (Fig. 8A) and eugenol treated group (Fig. 8C). In contrast, it was demonstrated that arsenic trioxide induced histopathological changes in the renal tissues, such as the destruction of tubular structures, necrosis, and disorganization, interstitial fibrosis (Fig. 8B). However, co-treatment with eugenol significantly diminished arsenic induced histological alterations (Fig. 8D).

Discussion

An outstanding benefit of As_2O_3 for APL is due to its ability to the degradation of PML/RAR alpha in the oncoprotein of acute promyelocytic leukemia (Zhang et al., 2010). However, the therapeutic use of As_2O_3 is limited by its toxic side effects in various organs.

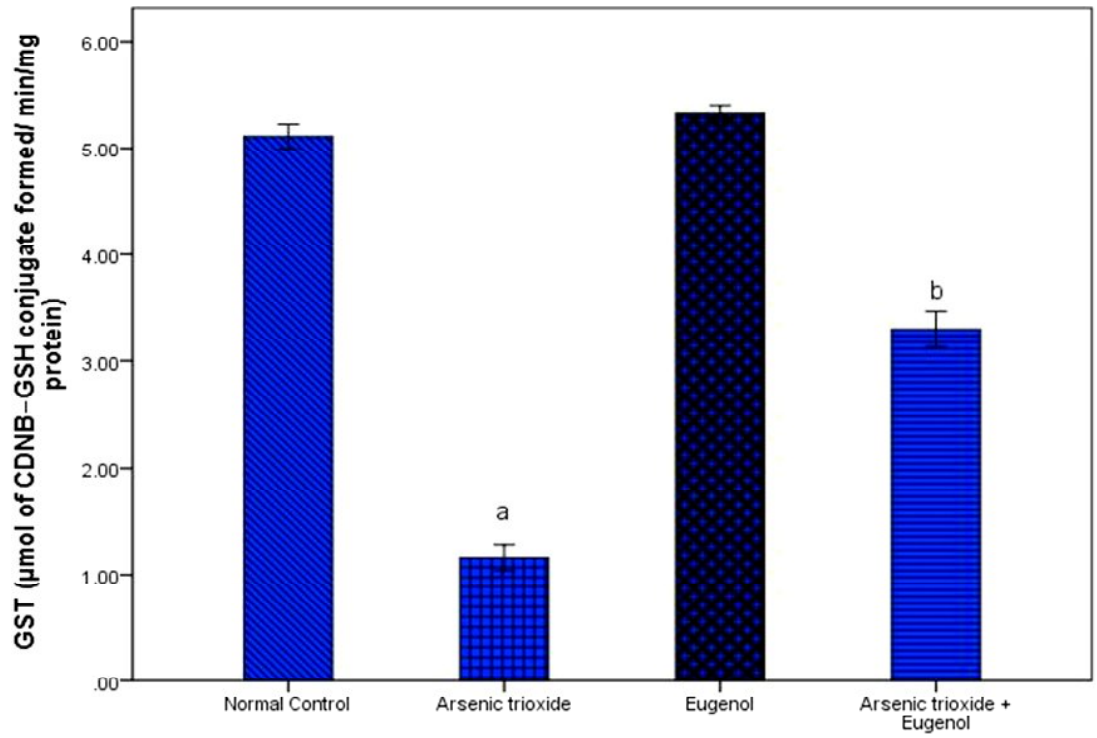

Fig. 3 : Effect of eugenol on glutathione –s-transferase level in renal tissue. Experiment consist of Normal control, Arsenic trioxide – 4 mg/ kg body weight, Eugenol – 5 mg/kg body weight, and Arsenic trioxide – 4 mg/kg body weight) + Eugenol – 5 mg/ kg body weight. Data represented as mean±SD, n=6. 'a' (significant difference between Normal control and Arsenic trioxide), 'b' (significant difference between Arsenic trioxide and Arsenic trioxide + Eugenol). P<0.05.

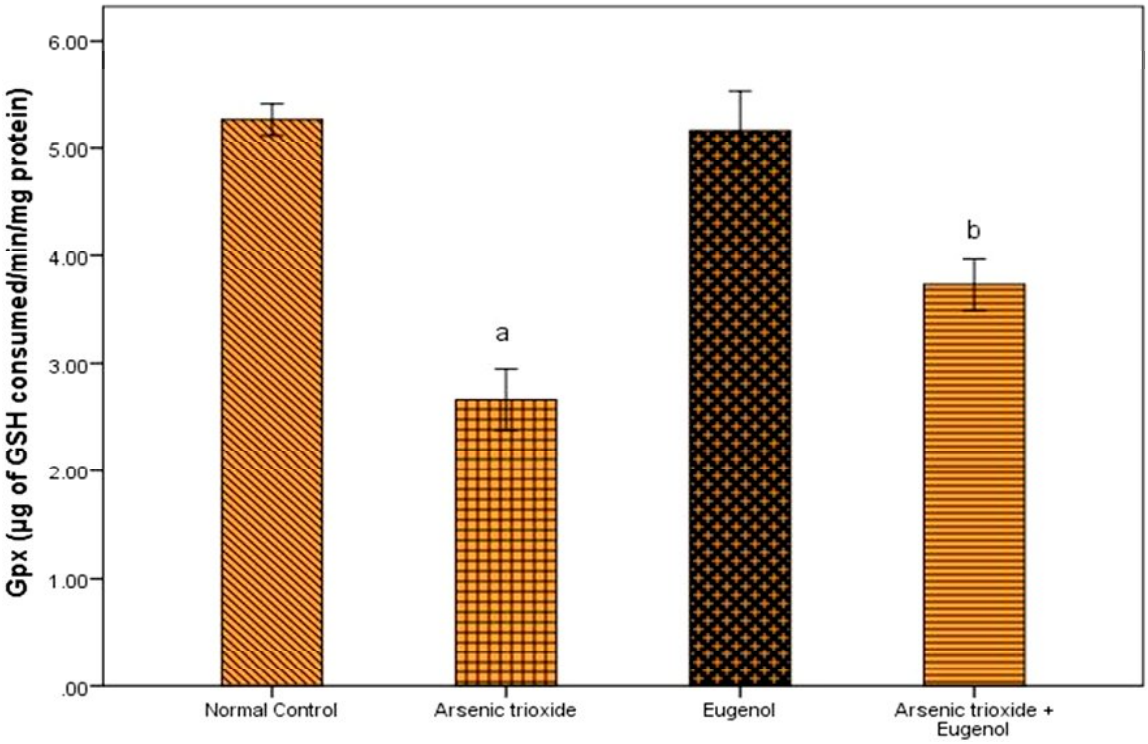

Fig. 4 : Effect of eugenol on renal tissue glutathione peroxidase activity. Experiment consist of Normal control, Arsenic trioxide – 4 mg/kg body weight, Eugenol – 5 mg/kg body weight, and Arsenic trioxide – 4 mg/kg body weight) + Eugenol – 5 mg/kg body weight. Data represented as mean±SD, n = 6. 'a' (significant difference between Normal control and Arsenic trioxide), 'b' (significant difference between Arsenic trioxide and Arsenic trioxide + Eugenol). P<0.05.

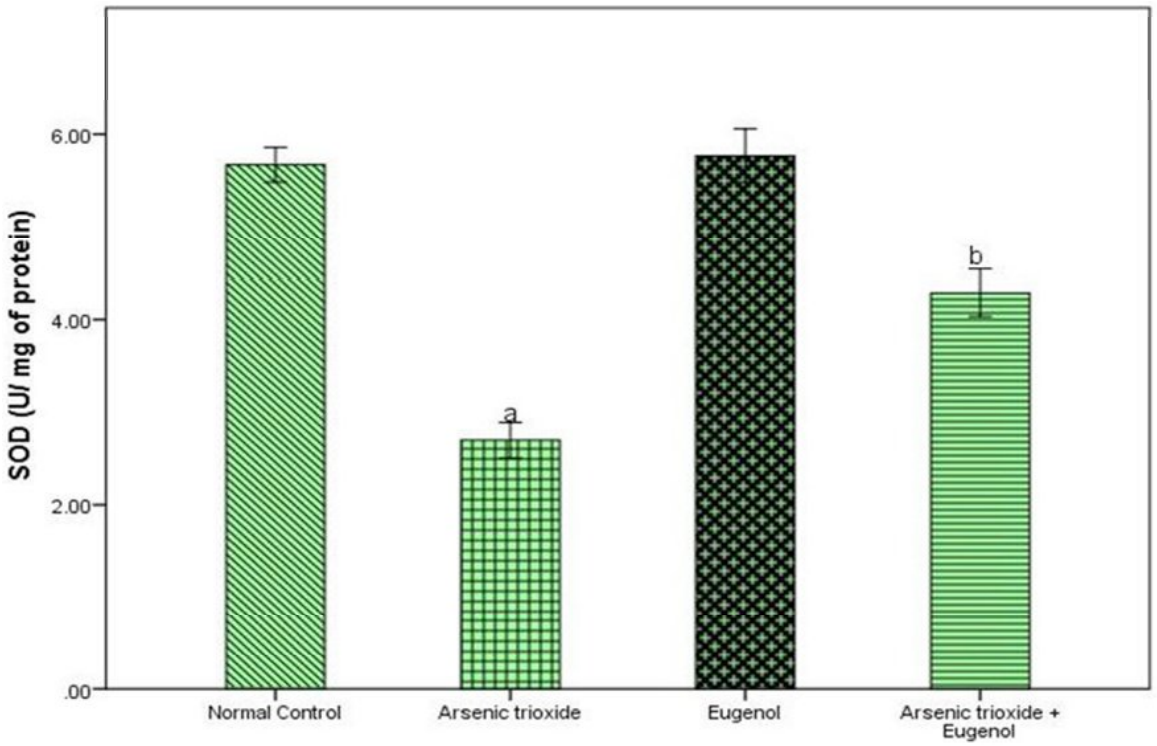

Fig. 5 : Effect of eugenol on superoxide dismutase activity in kidney. Experiment consist of Normal control, Arsenic trioxide – 4 mg/ kg body weight, Eugenol – 5 mg/kg body weight, and Arsenic trioxide – 4 mg/kg body weight) + Eugenol – 5 mg/kg body weight. Data represented as mean±SD, n = 6. 'a' represents the significant difference between Normal control and Arsenic trioxide), 'b' represents significant difference between Arsenic trioxide and Arsenic trioxide + Eugenol). P< 0.05.

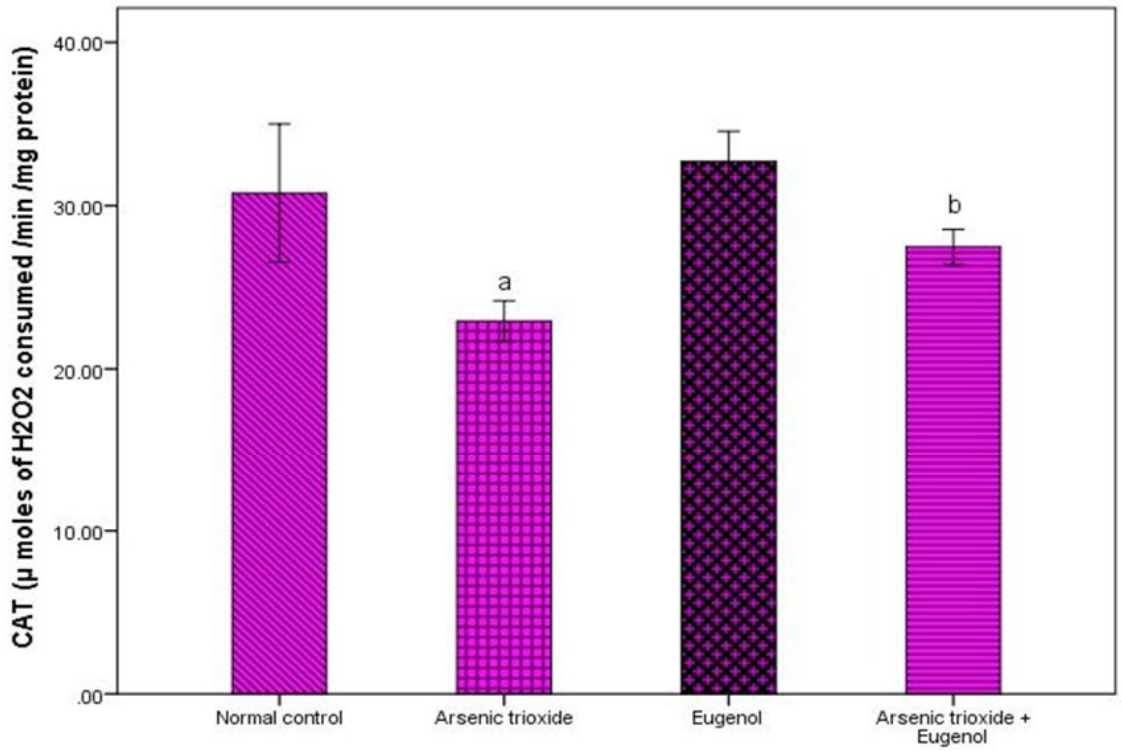

Fig. 6 : Effect of eugenol on enzymatic antioxidant catalase activity in renal tissue. Experiment consist of Normal control, Arsenic trioxide – 4 mg/kg body weight, Eugenol – 5 mg/kg body weight, and Arsenic trioxide – 4 mg/ kg body weight) + Eugenol – 5 mg/ kg body weight. Data represented as mean±SD, n = 6. 'a' represents the significant difference between Normal control and Arsenic trioxide), 'b' represents significant difference between Arsenic trioxide and Arsenic trioxide + Eugenol). P<0.05.

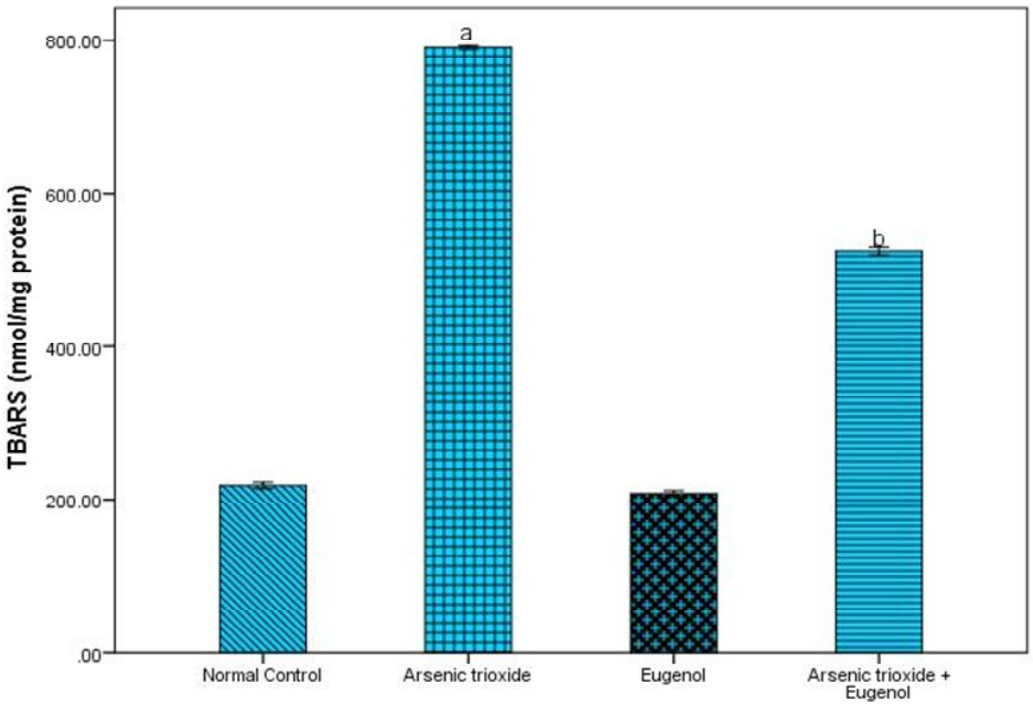

Fig. 7 : Effect of eugenol on lipid peroxidation in renal tissue. Experiment consist of Normal control, Arsenic trioxide – 4 mg/ kg body weight, Eugenol – 5 mg/kg body weight, and Arsenic trioxide – 4 mg/kg body weight) + Eugenol – 5 mg/kg body weight. Data represented as mean±SD, n = 6. 'a' represents the significant difference between Normal control and Arsenic trioxide), 'b' represents significant difference between Arsenic trioxide and Arsenic trioxide + Eugenol). P<0.05.

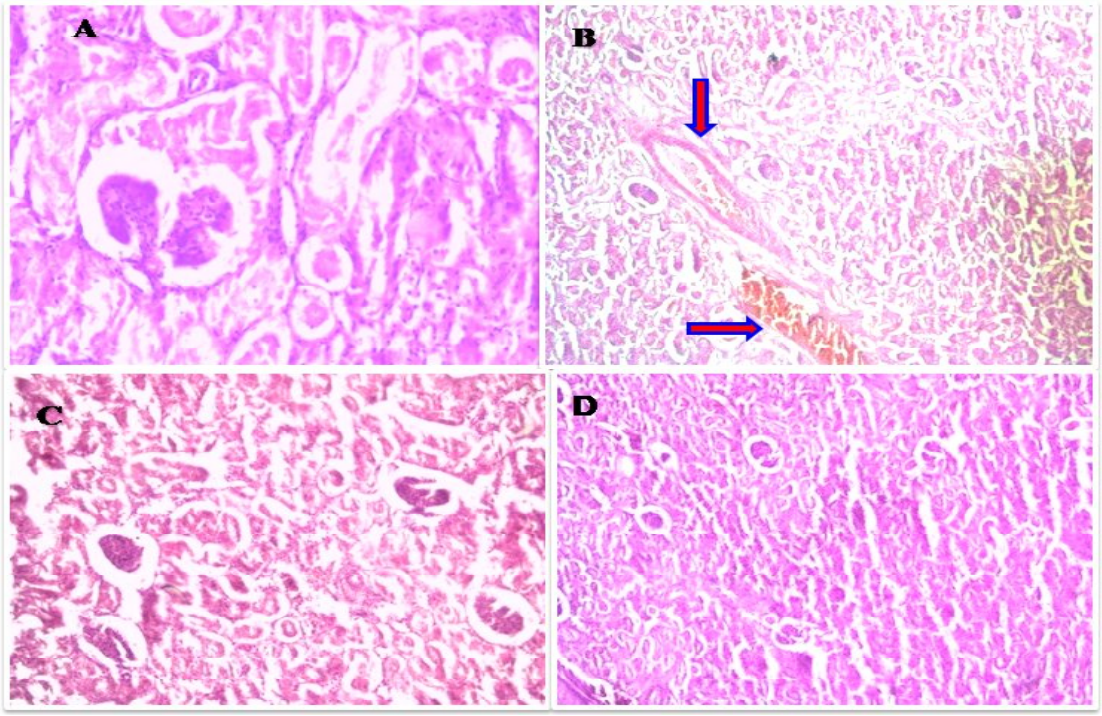

Fig. 8 : Effects of eugenol on arsenic trioxide induced oxidative stress in the histology of renal tissue. (A) – Normal rat kidney (H&E100X). Intact glomeruli in the cortical region with patent tubular epithelium, glomeruli and tubules were normal. (B) – Arsenic trioxide (4 mg/ kg body weight) treated rat kidney (H&E 100X). Showing proximal and distal tubular necrosis, tubular degeneration, tubular dilation, thickened basement membrane and marked atrophy of many glomeruli cells. (C) – Eugenol (5 mg/kg body weight) treated rat kidney (H&E 100X) showing normal renal histoarchitecture with glomeruli and renal tubules. (D) – Arsenic trioxide + Eugenol (4 mg/kg body weight +5 mg/kg body weight)-treated rat kidney (H&E 100X) Atrophy of glomerular tufts accompanied by moderate necrosis and congestion, degeneration and desquamation of tubular epithelium.

Therefore the development of strategies to reduce these toxic effects would allow us to explore the full therapeutic potential of As_2O_3 in cancer therapy. In this study, our goal was to investigate the renal protective effects of eugenol, a naturally occurring monoterpenoid in combination with APL drug arsenic trioxide in Wistar rats.

The tissue uptake of arsenic was analyzed by inductively coupled plasma emission spectroscopy (ICP-OES). Arsenic trioxide treated rats has shown deposition of arsenic in renal tissue. During chronic exposure, arsenic is known to accumulate in the liver, kidneys, heart, spleen, and muscles (Ratnaike et al., 2003). Emadi and Gore (2010) reported that arsenic compounds are cytotoxic and cause renal tissue damages. In addition to this kidney is sensitive to arsenic because it has an inefficient oxidative system (Prozialeck et al., 2007). We observed the administration of eugenol with arsenic trioxide prevent or reduce the accumulation of arsenic trioxide in the kidney.

Arsenic administration caused marked renal dysfunction as evidenced by the significant increase in blood urea, serum creatinine, and GGT levels. As_2O_3 has been reported to cause renal injury, elevated serum creatinine (CREA) and blood urea nitrogen (BUN) levels in clinical studies (Sakurai et al., 2006). Urea is the major nitrogen containing the metabolic product of protein metabolism. An elevated level of blood urea is known to be correlated with increased protein catabolism. The elevation in the levels of BUN and CREA in the serum of As_2O_3 treated rats is considered to be an important marker of renal dysfunction (Augusti et al., 2008). Vaezi et al (2017) have reported arsenic trioxide related renal toxicity in mice. Combination therapy with eugenol protected the kidney function from arsenic intoxication as indicated by significant restoration of serum and blood urea, creatinine and GGT as well as creatinine clearance rate.

The intra cellular antioxidant system comprises of different free radical scavenging antioxidant enzymes along with some non-enzymatic antioxidant like GSH and thiols (Fatma et al. 2009). Thus to eliminate free radicals, these antioxidant play a key role and maintain an equilibrium with oxidants and reductants. In the present study, arsenic treatment resulted in a dramatic decline in the level of GSH, GST, GPx, SOD, and CAT. In a study by Sener et al (2005) observed ROS generated in tissues are normally scavenged by enzymatic and non-enzymatic antioxidants. The non-enzymatic antioxidant GSH has a crucial role in cellular defense mechanisms against toxicity and oxidative stress resulting from exposure to arsenic (Chen et al., 2012). The reduced level of GSH in our study also agree with the observations of Akhand et al (2004) that arsenic is known to be a potent sulfhydryl reactive chemical capable of binding with proteins. Jones (2002) reported that GSH with its sulfhydryl group functions in scavenging of free radicals as well as xenobiotics. Our results substantiated with the observations of Srivastava and Shivanandappa (2010) that GSH depletion leads to lowered cellular defense mechanism. While GST activity appears to be linked to the activity of GSH (Hayes et al., 2005) and the declined level of GST activity may be due to the deficiency in GSH level. In the present study arsenic intoxication also significantly reduced the activity of GPx. GPx is an enzymatic antioxidant play an important role in the elimination of H_2O_2 and lipid hydroperoxide using GSH as a hydrogen donor (Lubos et al., 2011). Ekor et al (2006) observed that intracellular SOD and CAT enzymes are prominent in the antioxidant system that catalyzes the dismutation of superoxide radicals ($.O^{2-}$) to hydrogen peroxide, and decomposition of H_2O_2 to H_2O respectively. Furthermore, Yamanaka et al (1991) demonstrate that in arsenic metabolism one electron reduction of molecular oxygen by dimethyl arsenic produces superoxide anion radicals. SOD is a primary defensive enzyme and prevents the further generation of free radicals (Yamanaka et al., 2004). Decreased SOD activity in organs suggests that the accumulation of superoxide anion radical, which would leads to a raised level of hydroperoxides along with the lowered activity of CAT and GPx (Mahza and Shivanandappa, 2013). The recent study report from Nasiry et al (2017) well established renal toxicity related antioxidant depletion by arsenic trioxide in rats. Our findings indicate combination treatment with eugenol, a naturally occurring antioxidant would scavenge the free radical produced by arsenic and this might be

the reason of enhanced antioxidant status. It has been reported that phenolic antioxidants, including eugenol, has powerful free radical scavenging effect (Binu and Harikumaran Nair, 2015). The allyl group in the phenolic rings of eugenol is responsible for radical scavenging effect through the mechanism of electron spin resonance (Kuhn and Winston, 2007).

Eugenol interferes with initiation as well as propagation of lipid peroxidation and it is attributed to the free radical scavenging effect of eugenol (Nagababu and Lakshmaiah, 1994). The experimental rats administered with arsenic trioxide showed a rise in membrane peroxidation rates compared to normal control rats. Earlier studies proved that the compound arsenic increases lipid peroxidation and suppresses antioxidants in the kidney (Mittal and Flora, 2006; Nandi et al., 2006). Manimaran et al (2010) have reported that kidney is relatively more susceptible to oxidative stress induced by arsenic. Malonyldialdehyde (MDA) levels, an indicator of free radical generation and it formed as a breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids (Draper et al., 1990). Wang et al (2006) observed the role of MDA in assessing oxidative stress and different types of biological damages. Co-treatment with eugenol reduces the lipid-peroxidation which could be agreed with the report by Nagababu and Lakshmaiah (1994) that eugenol has significantly decreased the rate of lipid-peroxidation. Murakami et al (2005) also reported the effect of eugenol on lipid peroxidation and oxidation of low-density lipoproteins.

Our results showed increased lipid peroxidation in the kidney of arsenic treated rats which are associated with renal damage. Histopathological examination of renal tissue of arsenic trioxide treated rats showed the destruction of the tubular structure, necrosis, disorganization and interstitial fibrosis. These findings are in agreement with previous reports of Emadi and Gore (2010). Arsenic trioxide produces ROS, which may act as a signaling molecule in the reduction of oxidative stress and tissue injury (Yu et al., 2013). However, co-treatment with eugenol could prevent the changes and could also maintain the ultra structure almost similar to that of normal controls. The nephroprotective effects of eugenol can be partially attributed to the properties that scavenge free radical activity and enhance the antioxidant defense system.

In this study, eugenol has shown a protective action against antileukemic drug arsenic trioxide induced oxidative stress to the renal tissue as evidenced by the lowered urea, uric acid, creatinine and GGT enzyme activities and elevated levels of the enzymic and non-enzymic antioxidants along with creatinine clearance rate. In addition, lowered rate of lipid peroxidation decreased arsenic accumulation and renal tissue damage in the kidney demonstrated that eugenol offers protection against arsenic. The facts that eugenol is an antioxidant, and that it prevents arsenic induced toxicity *in vivo* make the present conclusions and provide an impetus for further studies.

Conflict of Interest

The authors declare that there is no conflict of interest involved in this study.

Acknowledgments

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