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Research Article

Therapeutic potential of *P. santalinus* against alcohol-induced histo-pathological changes and oxidative damage in heart and lungs

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ABSTRACT

Alcohol consumption is the third leading cause of global deaths due to Alcoholic Liver Disease (ALD) and Coronary Heart Disease (CHD), accounting for 4.5% of total deaths. The present investigation aimed at forwarding *P. santalinus* heartwood extract (PSE) as a potential therapeutic agent to alleviate alcohol-induced oxidative stress and tissue damage. In this study male albino Wistar rats were treated with 20% alcohol (5g/kg b.wt/day) and PSE (250mg/kg b.wt/day) for 60 days. Results showed that chronic alcohol administration significantly (P<0.05) increased lipid peroxides and nitric oxide (NOx) levels in heart and lung tissues, surprisingly these levels were brought back close to normal level by PSE administration to alcohol administered rats. Moreover, alcohol administration decreased the content of reduced glutathione (GSH) and activities of glutathione peroxidase (GPx), glutathione-s transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) in heart and lung, which were significantly raised to normal level by the administration of PSE. Phytoextracts contain several active components acting on different potential molecular targets/sites at a time rendering protection and ameliorating alcoholic damage or toxicity and/or by reducing the burden of alcohol related diseases and risk. Furthermore, alcohol induced tissue damage mitigation by the PSE was confirmed by histopathological restoration in heart and lung. Multiple phytocompounds like santalins, lignans, lupeol, pterostilbenes present in PSE might have shown protection against alcohol-induced damage by exhibiting strong free radical scavenging activity and acting at different signalling mechanisms or modulating the factors involved in gene expression.

Keywords: Alcohol; Heart; Lung; Oxidative stress; *P. santalinus.*

INTRODUCTION

Excessive alcohol consumption is global problem resulting in millions of deaths including hundreds of thousands of young lives and is reported as the third leading cause of deaths world-wide (WHO, *2014*). Chronic alcohol consumption is a risk factor for several diseases and disorders among which alcoholic liver disease (ALD), coronary heart disease (CHD), accute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD) including several organ cancer (Barclay *et al., 2008*; Ghosh *et al., 2012*). Alcohol affects virtually every organ in the body with no exception by exerting multi-factorial actions on cellular and molecular functions. It is well established that alcohol interfering some mechanisms are common to multiple tissues and others are tissue specific (Dinu *et al., 2005*; Jung *et al., 2011*). As people are addicted to alcohol consumption and are unable to quit it, the best way is to reduce the burden of alcohol related diseases and deaths is the development of therapeutic strategies. Cells are naturally equipped with antioxidant defenses to counterbalance free radical production. Overproduction of free radicals is one of the reasons for a variety of diseases. The current investigation was planned to evaluate chronic alcohol-induced oxidative stress in the heart and lung tissues of rats and to explore the ameliorative effect of P. santalinus heartwood extract (PSE).

Earlier studies clearly revealed that excessive chronic alcohol consumption predisposes humans and animals to several clinical problems such as atherosclerosis and other risks such as angina, peripheral cardiovascular disease and several other cardiovascular problems like vaso motor dysfunction and inflammation (Castnazo *et al., 2010*). Modification of lipids is integral components for initiation and progression of atherosclerosis in part by its effects on lipid profile. Chronic alcohol consumption results in multiple complex changes in the lung. Ultimately, these changes have serious consequences when a second insult, such as pneumonia or sepsis occurs resulting in an increased incidence of acute respiratory distress syndrome (ARDS) (Ischaki *et al., 2008*).

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Down et al. (2013) described the changes associated with alcohol induced acute respiratory distress syndrome and alcohol induced distal lung problems in animals and humans.

Pterocarpus santalinus, is a small to medium sized deciduous tree belong to Fabaceae family. It is widely distributed in the tropical regions of the world, especially in India, Sri Lanka, Taiwan and China (Navada and Hari, *2014*). *P. santalinus* extract showed anti-microbial (Manjunatha, 2006a), antioxidant (Wu *et al., 2011b*), hepato-protective (Dhanabal *et al., 2007*), anti-diabetic (Kondeti *et al., 2010*), gastro-protective (Narayana *et al., 2005*) and wound healing mechanisms (Kumar, *2011*). Also available literature demonstrated the presence of several phytocompounds in heartwood powder in particular santalin A, B and Y, pterocarpol, pterocarptriol, isopterocarpalone, pterocarpodiolone, cryptomeridol and several non-specific compounds such as isoflavones, isoflavonoid glucosides, triterpenes, sesquiterpenes, β-sitosterol, lupeol, epicatechin, lignans and pterostilbeans (Cho *et al., 2001*; Kesari *et al., 2004*). Since, it is a valuable source of various multiple phyto-compounds, we hypothesized that *P. santalinus* can be used as an effective therapeutic agent against various ailments. Hence, the present study was designed to study the therapeutic potential of *P. santalinus* against alcohol-induced oxidative/nitrosative stress in heart and lung tissues of rats.

MATERIALS AND METHOS

1. Preparation of extract

Heartwood powder of *P. Santalinus* was purchased in local market and was used to prepare *P. santalinus* heartwood extract (PSE). Heartwood powder was placed in mixture of methanol and sterile distilled water (80:20, V/V) for 48 h and the mixture was thoroughly stirred until extract has been dissolved. The mixture was then centrifuged at 2500 rpm for 10 min and the supernatant was filtered and evaporated to dryness at 45ºC with a rotary evaporator. The extract was dissolved in distilled water and then used for experimentation.

2. Animals

Two month old male albino Wistar rats weighing 120-140 g were procured from Sri Venkateswara agencies, Bangalore, India. Animals were maintained on a standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* with 24h light-dark cycle in University animal house. After acclimatization for a week, animals were divided into four groups (n=8) viz., group-I, served as controls, group-II, alcohol, group-III, PSE alone and group-IV, alcohol and PSE. Alcohol (20%) was administered at a dose of 5g/kg b.wt/day, PSE was administered at a dose of 250 mg/kg b.wt/day. Control rats received iso-caloric glucose instead of alcohol. All treatments were given orally using intubation tube for a period of 60 days. Experimentation and animal maintenance were done with prior approval of institutional animal ethics committee. At the end of experimental period the animals were fasted overnight and sacrificed by cervical dislocation. Immediately blood was collected into heparinised tubes by cardiac puncture and plasma was separated by centrifugation at 3000 rpm for 10 min. Lung and heart tissues were collected and stored at -80°C until assays were carried out. A part of heart and lung tissue was fixed in 10% neutral formalin solution for histo-pathological analysis.

3. Measurement of lipid peroxides and nitric oxide levels

Lipid peroxidation extent was measured both in heart and lung as described previously (Ohkawa *et al., 1979*). Total NOx in the form of nitrite and nitrate levels were measured by the method of Sastry *et al* (Sastry *et al., 2002*).

4. Assays of enzymic and non-enzymic antioxidants in heart and lung

Heart and lung tissue were homogenized (10% w/v) in tris buffer (0.1M, pH 7.4), centrifuged (10,000xg for 20 minutes at 4ºC) and the supernatant was used for all the biochemical parameters. Total glutathione (GSH) content was measured by the method of Ellman's (Ellman, 1959) and the activities of glutathione reductase (GR) (Pinto and Bartely, 1969), glutamine-s-transferase (GST) (Rotruck *et al., 1973*), glutathione peroxidise (GPx) (Rotruck *et al., 1973)*, catalase (Aebi, 1984) and superoxide dismutase (SOD) (Marklund and Marklund, 1974) were determined. Protein concentration was determined by the method of Lowry *et al* (Lowry *et al., 1951*).

5. Histo-pathological studies

A portion of heart and lung tissues were dissected and fixed in 10% neutral buffered formalin solution for 24 h as described by Raghuramulu *et al* (1983). The fixed tissue was processed routinely, and then embedded in paraffin, sectioned to 3-5 µm thickness, deparaffinised and rehydrated using standard techniques. Morphological changes in both heart and lung sections were observed by hematoxylin and eosin, original magnificationx100

6. Statistical analysis

Data were subjected to statistical analyses values are mean ± SD of 8 rats in each group. Student t-test followed by Duncan's Multiple Range (DMR) test followed by one-way ANOVA was performed to find out significant difference between groups. A $p < 0.05$ was considered statistically significant.

RESULTS

TBARS levels were used as an index of lipid peroxidation, in the present study heart and lung TBARS data were presented in Fig. 1(a) and (b). Administration of

PSE to alcohol administered rats significantly ($p < 0.05$) prevented the elevation in TBARS level in comparison with alcohol alone administered rats.

Nitrite and nitrate levels are an index of NOx production. NOx levels were measured in heart and lung tissues and the data were presented in Fig. 2(a) and (b). Alcohol administered rats showed significant increase in heart and lung tissue NOx levels. However, administration of PSE to alcohol administered rats significantly prevented the elevation of NOx levels in both heart and lung.

The effect of PSE on enzymic and non enzymic antioxidants in alcohol administered rats were summarised in Table 1 and 2. The activities of antioxidant enzymes *viz*., GPx, GST, GR, SOD, catalase and the content of GSH was markedly decreased in heart and lung tissues of alcohol administered rats in comparison to the other experimental groups. Treatment of PSE to alcohol administered rats significantly ($p < 0.05$) increased these levels close to normal levels.

Alcohol-induced histological changes in heart and lung tissue were presented in Fig 3 & 4. Microscopic examination of heart and lung sections of alcohol fed rats showed degeneration of tissue and necrotic changes with distortion of normal architecture, prominence and widening of heart sinusoids were observed in comparison with other experimental groups. Administration of PSE to alcohol administered rats rectified these histopathological alterations in both heart and lung.

DISCUSSION

Alcohol toxicity is not often limited to a single tissue, for example a fractured bone may also be associated with skin and muscle or tendon damage. It is well known that alcohol cardiomyopathy (ACM) is characterized by dilated left ventricle normal or reduced left ventricle wall thickness and increased left ventricle mass leading to heart failure. Several studies demonstrated ethanol induced myocardial dysfunction with reduced cardiac contractility, enlarged cardiomyocyte, mitochondrial damage, and apoptosis (Laonigro *et al., 2009*). Alcohol consumption affects the lung airways functions as alcohol moves from bronchial circulation across the air way epithelium and into the conducting air ways of the lung. Reports revealed that moderate or mild concentrations of alcohol may enhance muco-ciliary clearance, stimulates broncho dilation and probably attenuates the air way inflammation and injury observed in asthema and chronic obstructive pulmonary disease (COPD) (Yeliger *et al., 2012*). Prolonged and heavy exposure of alcohol impairs muco-ciliary clearance, may complicate asthma management and worsens in COPD patients. Alcohol has been suggested to be a trigger of asthma and also treatment of asthma (Sisson *et al., 2007*). Das and Mukharjee suggested that long term ethanol administration aggravates systemic and local oxidative stress which may be associated with lung tissue injury (Das and Mukherjee, 2009). Research focused on the mechanism of alcohol mediated changes in air way functions has been identified that mediate alcohol effects within the lung air ways. These include prominent roles for the second messengers, calcium and NOx, regulatory kinases including PKG and PKA, alcohol-and aldehyde metabolizing enzymes such as ALDH2 (Roman *et al., 2005*).

Alcohol induced generation of free radicals initiate lipid peroxidation which in turn provides supply of more number of free radicals in an amplified way leading to oxidative damage of each tissue and organ. Moreover alcohol induced generation of NOx is a characteristic response in alcohol toxicity. Nitrite and nitrate, the end products of NOx metabolism are reliable indices of NOx production. Much of the toxicity of NOx may be mediated by forming more potent peroxynitrite (O_2+ NO→$NOONOO^-$). Peroxynitrite formation also participates in ethanol induced oxidative stress. Since NOx can diffuse freely into the mitochondria, nitric oxide rapidly reacts with mitochondrial superoxide (Radi *et al., 2002*; Das and Vasudevan, 2007). In the present study, nitrite and nitrate levels in tissues like heart and lung were increased in rats receiving alcohol when compared to Controls, However, these levels were restored to normal by PSE supplementation to alcohol administered rats. Elevated levels of NOx in heart and lung might be due to increased iNOS expression. The phytocompounds in PSE possess significant antioxidant activity and are able to reduce lipid peroxidation making it suitable for application in food and drug products. However, the precise mechanisms related to the chemical structures of specific and non-specific phytocompounds and their functional relation with Respect to biomolecules require an in-depth study.

Chronic alcohol consumption could impair enzymatic and non-enzymatic antioxidant system that protects cells against ROS mediated damage. Endogenous antioxidant enzyme system involved in ROS elimination include SOD, CAT, GST and GPx serves as the first line of defense against oxidative damage. Non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin-C and Vitamin-E protecting the cell against lipid peroxidation in biological system (Shanmugam *et al., 2011*). Several crude extracts or isolated compounds from medicinal plants have been shown to enhance the activities of endogenous enzymatic Moreover, several herbal extracts could reverse the elevation of MDA levels induced by chronic alcohol administration.

Glutathione (GSH), a tripeptide, is a non-protein foot marker of oxidative stress and plays a critical role in maintaining defense against oxidants. Glutathione the most abundant non protein thiol in cells plays a role in disposition of ROS (Brown *et al., 2004*). The GSH levels in alcohol treated rats were reported to be decreased by several researchers (Mallikarjuna *et al., 2008*). In the present study, heart and lung GSH content significantly decreased in alcoholic rats, however PSE supplementation to alcohol fed rats showed restored GSH

Table i: Effect of PSE on heart antioxidant status in alcohol fed rats.

Parameter	C	A	PSE	A+PSE
GSH	3.6 ± 0.13	1.9 ± 0.18*	3.6 ± 0.13	3.5 ± 0.08ns
GPx	7.2 ± 0.18	3.9 ± 0.30*	7.1 ± 0.30	6.9 ± 0.10ns
GR	30.4 ± 0.74	20.6 ± 0.66*	30.3 ± 0.43	28.5 ± 0.31ns
GST	67.6 ± 0.50	58.2 ± 0.44*	66.7 ± 0.44	66.5 ± 0.30ns
SOD	23.9 ± 0.50	13.6 ± 0.32*	23.8 ± 0.40	22.7 ± 0.22ns
CAT	9.3 ± 0.12	4.2 ± 0.09*	9.1 ± 0.13	8.9 ± 0.14ns

GSH is expressed as µg/mg protein and remaining values as µmole/min/mg protein and are represented as the mean ± SD. A $p < 0.05$ is considered as significantly different between groups. Asterisk"*" indicates significant from controls, "ns" indicates not significant from controls and PSE alone administered rats.

Table ii: Effect of PSE on lung antioxidant status in alcohol fed rats

Parameter	C	A	PSE	A+PSE
GSH	9.2 ± 0.5	4.1 ± 0.48*	9.2 ± 0.20	8.9 ± 0.60 ns
GPx	6.7 ± 0.6	2.2 ± 0.06*	6.6 ± 0.14	5.9 ± 0.08ns
GR	16.4 ± 1.4	9.8 ±1.11*	16.5 ± 1.17	15.7 ± 1.35 ns
GST	26.9 ± 1.3	16.7 ± 1.14*	26.7 ± 0127	24.4 ± 1.19 ns
SOD	1.7 ± 0.04	0.9 ± 0.02*	1.7 ± 0.10	1.5 ± 0.05 ns
CAT	3.3 ± 0.12	2.2 ± 0.05*	3.5 ±0.07	2.9 ± 0.04ns

GSH is expressed as µg/mg protein and remaining values as µmole/min/mg protein and are represented as the mean ± SD. A $p < 0.05$ is considered as significantly different between groups. Asterisk"*" indicates significant from controls, "ns" indicates not significant from controls and PSE alone administered rats.

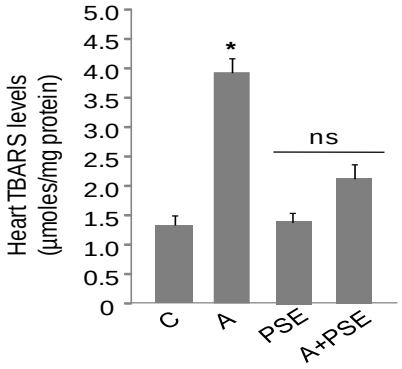

Figure 1(a): Effect of PSE administration on heart TBARS levels in alcohol administered rats.

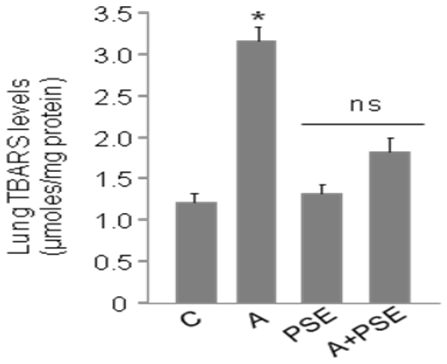

Figure 1 (b): Effect of PSE administration on lung TBARS levels in alcohol administered rats.

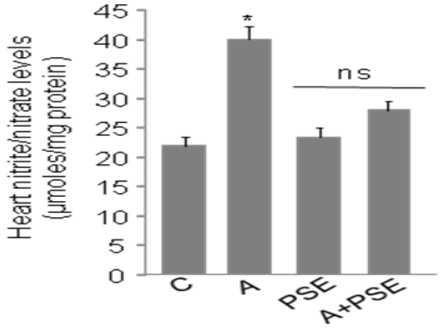

Figure 2 (a): Effect of PSE administration on heart nitrite/nitrate levels in alcohol administered rats.

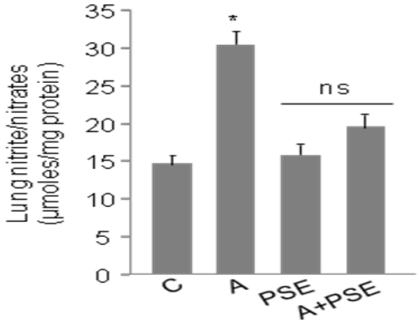

Figure 2 (b): Effect of PSE administration on lung nitrite/nitrate levels in alcohol administered rats.

Figure 1(a), (b), 2(a), (b): Values are represented as the mean ± SD (n=8). A $p < 0.05$ is considered as significantly different between groups. Asterisk"*" indicates significant from controls, "ns" indicates not significant from controls and PSE alone administered rats.

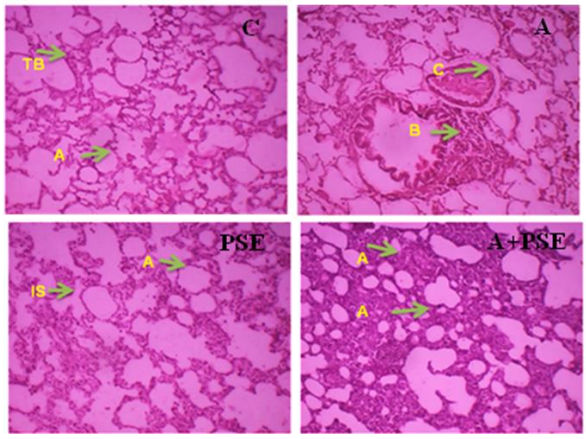

Figure 3: Histological micrograph of lung sections of rats stained with haematoxylin and eosin.
TB: Terminal Bronchiole; **A:** Alveoli; **IS:** Inter alveolar Septum; **B:** Bronchiole; **C:** Congestion

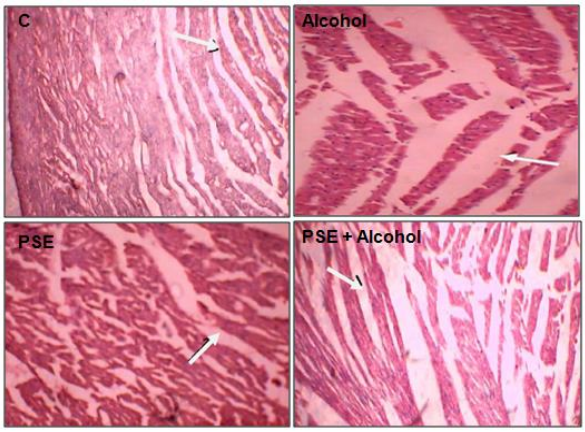

Figure 4: Histological micrograph of heart sections of rats stained with haematoxylin and eosin.

content. SOD stand in first line of defense and is the powerful antioxidant enzymes protects cells against the deleterious effects of oxygen radicals in the cells. In the present study, the SOD activity was significantly decreased in heart and lung tissues of alcohol administered rats. The SOD activity was restored near to normal levels by the PSE administration. Catalase acts as a preventive antioxidant and plays an important role in the protection against the deleterious effects of LPO. Significantly decreased catalase activity of heart and lung was due to exhaustion of enzymes as a result of oxidative stress caused by alcohol. Presumably, a decrease in CAT activity could be attributed to cross-linking and inactivation of the enzyme protein in the lipid peroxides. The CAT activity was restored to normal after the treatment with PSE. Glutathione peroxidase plays a pivotal role in H_2O_2 catabolism and detoxification of endogenous metabolic peroxides and hydroperoxides which catalyze reduced glutathione (GSH) level. The decreased level of glutathione peroxidase in the heart and lung of alcohol administered rats might be due to either free radical dependent inactivation of enzyme or depletion of its co substrates, GSH and NADPH. Decreased GPx activity after chronic exposure to alcohol was observed in our study. This finding is in agreement with earlier reports (Bailey *et al. 2001*). The decreased GR activity in heart and lung of alcohol administered rats might be result from this enzyme utilization to GSSG reduction or from NADPH deficiency. Although relatively resistant to spontaneous oxidation, GSH reacts rapidly and non-enzymatically with the hydroxyl radical, nitric oxide and peroxynitrite (Deneke, *2001*). Alcohol administered rats receiving PSE could effectively restored alcohol Induced oxidative damage in heart and lungs as evident from histopathological and biochemical results. The reversal of the GPx and GR activities in alcoholic rats receiving PSE can be attributed to the antioxidant activity and other detoxifying phytocompounds of PSE. Furthermore, the effect of PSE is more potent and multiple mechanisms involved in antioxidation, anti-inflammation, inhibition of lipid synthesis and increase of fatty acid β-oxidation and suppression of *de novo* lipid synthesis. This is possible by the integrated functionality as well as coordinated play by multiple principles present in PSE with chiefly certain specific and some non-specific compounds.

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