Alcohol-Induced Mitochondrial and NADPH Oxidase Mediated ROS Generation Alter Neuroendocrine Status: Role of *Pterocarpus Santalinus*

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Research Article

Keywords: Alcohol, Brain, Hormones, Oxidative stress, P.santalinus

Posted Date: October 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-945712/v1

Abstract

The association between oxidative stress and endocrine status with respect to the role of *Pterocarpus santalinus* (PSE) against alcohol-induced neurotoxicity in rats was investigated. Male albino rats were divided into, control, alcohol treated, alcohol + PSE treated, and PSE treated group. Twenty percent of ethanol (5g/kg body weight/day) was given with and without PSE (250 mg/kg body weight/day) for 60 days. Decreased plasma testosterone, estradiol, thyroid hormones (T_3 and T_4), and increased cortisol concentrations in alcohol treated rats were observed. Besides, elevated lipid peroxidation, protein carbonyls, NADPH oxidase (NOX), and cytochrome P-450 (CYP-450) activities, with mitochondrial dysfunction were noticed. Moreover, increased protein expression of the phosphorylated protein kinase C (p-PKC), phospholipase C (p-PLC), and NOX2 with decreased antioxidant status was also noticed in alcohol ingested rats. Administration of PSE to alcohol treated rats reduced oxidative stress by increasing antioxidant status, also modulated mitochondrial dysfunction and protein expression of p-PKC, p-PLC and NOX2. In conclusion, ROS generated *via* mitochondrial dysfunction causes activation of NOX activity through PLC and PKC dependent pathways leading to more ROS generation, which in turn alters the circulating hormonal levels. The phytocompounds present in PSE confer therapeutic efficacy by scavenging ROS and thereby offer protection.

Introduction:

Alcohol (ethanol) is most widely consumed all over the world. Chronic consumption of alcohol is a main health issue, causing organ toxicity leading to multiorgan pathologies (Wilson et al 2020).. Although a majority of the ingested ethanol is metabolized by the liver, it has intoxicating effects on the brain (Reddy et al. 2017). The central nervous system is influenced by alcohol reflecting the function, properties, pathologies leading to changes in behaviour and health of an individual. Besides, the brain exhibits more unwarranted effects due to chronic alcohol consumption. Moreover, the brain consumes/requires more oxygen, and abundant in total lipid content with less antioxidant enzymes as compared with other tissues, increased plasma membrane area to cytoplasm ratio, and expanded neuronal (axonal) morphology make the brain more vulnerable to oxidative stress (Perez et al 2020). Normal *cellular* metabolisms produce ROS (Reactive Oxygen Species). Mitochondrial electron transport chain, NADPH oxidases and, CYP450 activity are the major sites for endogenous ROS generation. Mitochondrial dysfunction and decreased antioxidant status in the brain of chronically alcohol-fed rats were noticed (Reddy et al. 2013). Moreover, alcohol-induced increased microsomal CYP450 activity elevates intracellular calcium levels (Harrah et al. 2007, 2008). NADPH oxidase is a membrane-bound enzyme complex and it requires translocation of subunits from the cytosol to membrane to form an active complex *via* protein kinase C and phospholipase C dependent pathway. Besides, alcohol, toxic metabolites of alcohol, acetaldehyde, increases oxidative stress and causes cells morphological changes and metabolic alterations (Waris et al. 2020). Increased oxidative stress in alcoholism initiates different cascades of events that influence the hormone homeostasis (Rachdaoui et al. 2013).

Endocrine secretions are chemical messengers produced and secreted in response to specific physiological and biochemical events and provoke a cascade of biochemical signals within the body which include alterations in enzyme expressions and activities, fluctuations in cell membrane and organelles composition, and may lead to change in behavior of cell type (Martin et al. 2019). Alcohol has numerous multifaceted functional regulation on hormonal function. Hypothalamus, positioned deep in the brain, is the key regulatory site of organ systems. Hypothalamus receives physiological and physical signals through nerve impulses and releases hormones in response to those signals (Marty et al.2020, Becker et al.2012). Alcohol interferes with the hormonal balance either directly by activating or inhibiting neuro-hormonal axes or indirectly by changing the metabolism of hormones (Wieczorek et al. 2015). Chronic alcohol consumption causes abnormalities in carbohydrate and lipid metabolism for which endocrine dysfunction might also play a role (Mullur et al.2014).

The available literature on ethnomedicinal information and our preliminary studies indicate the presence of several major bioactive components including, santalins A and B, pterolinus K and L, savinin, calocedrin, and pterostilbenes in *P. santalinus* heartwood aqueous extract (PSE) (Bulle et al. 2013a). Studies also reported beneficial health effects of PSE (Bulle et al. 2013b). In this study, we hypothesized that ROS produced by mitochondrial dysfunction and NADPH oxidase *via* PLC and PKC-dependent pathways might be playing a role in hormonal changes. We conducted the present study to verify the potential therapeutic efficiency of *P. Santalinus* in counteracting the alcohol mediated brain alterations on brain oxidative stress, antioxidant status and plasma hormones using alcohol-fed rat model.

Materials And Methods:

Preparation of extract

The aqueous extract was prepared from the heartwood powder of *P. Santalinus* as described previously (Bulle et al. 2013a). Before use, the extract was dissolved in water.

LC-MS analysis

Standardization of *Pterocarpus santalinus* extract was performed using LC/MS, Waters model -3100 coupled with LC-29960 system. The chromatographic separation was performed using Hypersil GOLD, THERMO C-18, 3µm, 4.6 X 50 mm. 0.1% formic Acid (1ml of formic acid in 1000 mL of HPLC grade water) and acetonitrile were used as mobile phase A and B respectively and used with a flow rate of 0.8 mL/min. The gradient elution started with 95% A/5% B 0-10 min. Photodiode array detector was set at 285 nm for acquiring chromatograms. Approximately 2mg of compound is weighed and dissolved in water for injections. The injection volume was 5 µL and peaks were monitored at 250 nm. A mass spectrum was recorded in positive ionization mode between *m/z* 100 to 800 as per the standard operating procedure and Electroscopy ionization (ESI) technique was used for the ionization of the sample.

Animals

Two-month-old male albino wistar rats (weight 120-140 g) were maintained on a regular rodent diet and water *ad libitum* at Sri Krishnadevaraya University vivarium. After adaptation for seven days, animals were divided into four groups (*n*=8).

1. Group I- Controls

2. Group II- 20% alcohol-treated

3. Group III - PSE-treated and

4. Group IV - 20% alcohol and PSE-treated.

Alcohol (20%) was administered at a dose of 5g/kg body weight per day, and PSE was administered at a dose of 250 mg/kg body weight per day. Iso-caloric glucose was administered to control rats. Alcohol and PSE were administered using an oral gavage feeding needle for 60 days. All animal studies were conducted following Institutional regulations with approved protocol from Sri Krishnadevaraya University institutional animal ethics committee (471/06/c/CPCSEA, dt.20th Sep 2016). After two months, the animals were fasted overnight and euthanized by cervical dislocation to collect blood (cardiac puncture) and tissues including the brain. Cortex was separated from brain and used immediately for analysis.

2.4.Measurement of TBARS, protein carbonyls, and NADPH oxidase activity:

Cortex was minced in tris-buffer (0.1M, pH 7.4, 10% w/v), centrifuged for twenty minutes (10,000xg for at 4ºC). The supernatant was separated and used for all the biochemical parameters. Thiobarbituric acid reactive substances (TBARS) levels were determined by measuring the formation of malondialdehyde as published earlier (Padmavathi et al. 2018). Protein carbonyls content was determined using a 2,4-dinitrophenylhydrazine (DNPH) assay (Reddy et al. 2011) NADPH oxidase activity levels were determined using cytochrome-c reduction assay (Yuan et al. 2013). The reaction mix was made in modified HEPES buffer (25mM, pH 7) and consisted of NADPH (100 mM), cytochome-C (150 mM) and membrane protein (100mg) with/without superoxide dismutase (200 units/mL). The activity (reduction of cytochome-c) was measured using a microplate reader (550nm). The activity levels of NADPH oxidase was determined with extinction coefficient of 21 mmol/ (L^{-}cm) and was presented in nmol/min/mg protein.

Measurement of CYP-450 activity

CYP-450 enzyme activity was determined as described earlier (Reddy et al. 2014). The CYP-450 content was measured using the extinction coefficient difference ($\Delta E_{450-490}$nm of 91 $cm^{-1}mM^{-1}$).

Measurement of T_3, T_4, TSH, Cortisol, Testosterone, and Estradiol Levels:

Concentrations of thyroid hormones (T_3, T_4, and TSH), testosterone, estradiol, and cortisol in plasma samples of alcohol administered rats and control rats were performed using chemiluminescence based

immunoassay system (Advia Centaur TM, Bayer Health Care Diagnostika, Vienna, Austria) (Padmavathi et al. 2015).

Histopathology:

Dissected brain tissue was fixed immediately in 10% neutral buffered formalin solution followed by embedding in paraffin. Five µm thickness sections were cut and conducted histopathological studies following standard methods. Hematoxilin and eosin staining were done to observe pathological changes.

Assay of non-enzymic and enzymic antioxidant status:

Total reduced glutathione content was determined using Ellman's reagent. Enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione-s-transferase (GST) and glutathione peroxidase (GPX) were measured as described before (Bulle et al. 2016a). Protein concentration was estimated by the method of (Lowry et. a 1951).

Measurement of mitochondrial complex activities

Complex-I (NADH-dehydrogenase), complex-II (succinate-dehydrogenase), complex-III (Cytochrome-*bc1*-complex/ubiquinol-ferricytochrome-*c*-oxidoreductase) and complex-IV (cytochrome-*c*-oxidase) activities were determined following published protocols (Remsberg et al. 2008, Fressco et al.2006, Faustino et al. 2019, Tiwari et al.2013).

Immunoblotting analysis

Brain tissue samples were cleaned, washed and grinded followed by sonication in RIPA lysis buffer with phosphatase inhibitors and protease inhibitors. The lysates were centrifuged at 14,000 rpm for 15min at 4^{o}C to collect the supernatant. Laemmli buffer was added to the lysates and electrophoresis (8%). The resolved proteins/lysates were PVDF membranes. Blocking of the membranes was done using 3% BSA followed by incubating with primary antibody (1:1000 dilution, overnight). Anti-total protein kinase C (Cat # 2056), anti-phospho PLCγ1(Tyr783) (Cat # 2821), anti-total PLCγ1 (Cat # 2822) and anti-pan PKCγ^{Thr514} (Cat # 9379) were purchased from Cell signalling Technology, USA. Anti-NOX 2 antibody (Cat # sc-74514, Santa Cruz Biotechnology, USA) and anti-α-tubulin (Cat # T-5168) were also used. Later blots were incubated with HRP conjugated secondary antibodies (1:10,000 dilution) and developed using an ECL solution. The images were captured using Alpha Innotech Imager.

Statistical analysis:

Statistical analysis was conducted using Student "t" test and one-way ANOVA. A p value, <0.01 and <0.05 were considered as significant.

Results:

The total ion chromatogram of *Pterocarpus santalinus* extract is shown in Fig. 1. LC-MS analysis of *Pterocarpus santalinus* extract revealed the presence of several bioactive compounds.

The effect of alcohol on TBARS and protein carbonyls levels in brain homogenates was shown in Fig. 1a and 1b. Alcohol-treated rats showed increased levels in TBARS and protein carbonyls compared to controls. Moreover, NADPH oxidase and CYP-450 activities were also measured and the data was presented in Fig. 1(c) and 1 (d) respectively. Compared to controls, higher NADPH oxidase activity and CYP-450 activity were observed in alcohol treated rats. PSE treatment prevented the elevation of TBARS, protein carbonyl levels and NADPX oxidase activity in alcohol treated rats significantly ($p < 0.05$) compared to alcohol-administered group. PSE treatment significantly decreased CYP-450 enzyme levels in alcohol-treated group. PSE alone administered control rats showed normal enzyme activity to that of controls.

Alcohol-induced histopathological alterations along with normal brain architecture were represented in Fig. 2. Degeneration and necrosis of brain tissue was observed in alcohol administered whereas control rats showed normal architecture with glial tissue.

Mitochondrial function was determined by measuring the activities of the electron transport complex. The activities of complex-I, III and IV (Fig. 3a, c, d) were significantly decreased in ethanol-fed groups compared to control rats. However, we did not observe any significant change in the levels of complex-II (Fig. 3b) in ethanol-fed rats compared to controls. Treatment of PSE to alcohol-treated rats normalized the activities of these complex-I, -III and -IV activities.

Effect of PSE on alcohol-induced alterations of total and phospho- PLC and PKC along with NOX2 protein expression were presented in Fig. 4. Results showed that alcohol administration significantly increased phosphorylated PLC and PKC protein expression. PSE treatment to ethanol-fed rats significantly ($p < 0.05$) prevented elevation of these phosphorylated proteins and NOX2 expression in alcohol administered rats.

Circulating levels of plasma T_3, T_4, TSH, testosterone, estradiol and cortisol levels were measured in all four rat groups and the values were furnished in Table 1. Concentrations of T_3, T_4, TSH, estradiol and testosterone were significantly decreased in alcohol administered rats with a significant increase in cortisol levels compared with control rats. Treating alcohol-fed rats with PSE decreased these hormone levels close to normal values. PSE alone administered rats did not show any significant changes compared to other experimental groups.

Table 2 shows the effect of PSE on enzymatic and non-enzymatic antioxidant levels in the rats. Brain homogenates of ethanol-fed rats showed decreased activities of SOD, GR, GST, GPX, and CAT followed by lesser content of glutathione when compared to the control group. Administration of PSE to ethanol-fed rats increased ($p < 0.05$) these enzyme activity levels and glutathione levels.

Discussion:

Chronic alcohol consumption creates multiple implications and ultimately impacts endocrine function for which several variables include the type of alcohol exposure/abuse including length and level of exposure. Other associated relevant conditions like hepatic dysfunction and nutritional abnormalities must be taken into account when assessing the hormonal status in alcoholics (Szkudelska et al. 2017). Previous studies showed that plant extracts and/or bioactive phytocompounds either individually or synergistically showed beneficial effects against many ailments (Howes et al. 2014). Multiple natural compounds showed beneficial effect on ROS inhibition and, enzymatic and non-enzymatic antioxidant defence at multiple levels including regulation of gene expressions, altering associated receptor functions, antioxidants and enzyme inhibitors (Seo et al. 2018). Our previous studies revealed that *Pterocarpus santalinus* extract offered protection against alcohol-induced oxidative stress in the liver and kidney by its strong free radical scavenging activity, the active phytocompounds present in *P.santalinus* might be acted potentially against alcohol-induced oxidative damage (Bulle et al. 2016a. 2016b). Moreover, alcohol consumption for longer periods causes hormonal imbalance, for which increased oxidative stress might be playing a role. So far no mechanistic study was carried out to study the association between increased oxidative stress and altered endocrine status in alcoholism. The present investigation describes the association between oxidative stress and endocrine status, also the modulatory role of *P.santalinus*. Previous studies reported the presence of stilbenes such as pterostilbene and resveratrol in the heartwood extract of *P. Santalinus (*Remsberg et al. 2008). Stilbenes are phenolics, composed of two aromatic rings (Fresscco et al.2006). These stilbenes are potential chemopreventive agents and also act as phytoestrogens protecting hormone-dependent tumor development (Faustino et al. 2019). Resveratrol possesses potential antioxidant, antibacterial, antiviral, anti-inflammatory, and anticancer activities (Tiwari et al 2013, Rauf et al. 2018). Another bioactive compound, β-sitosterol present in *P.santalinus* heartwood showed a cholesterol-lowering effect (Rudkowska et al. 2008). So, the current research was conducted to elucidate the molecular mechanisms of alcohol-mediated ROS production on endocrine status and further to verify the potential beneficial effects of PSE on alcohol-mediated ROS production, antioxidant status and associated hormonal levels.

Alcohol dehydrogenase metabolizes ethanol into acetaldehyde in the microsomes mediated by cytochrome P-450 2E1 (CYP2E1), and in the peroxisomes mediated by catalase (Teschke et al. 2018). Further oxidation of acetaldehyde to acetate involves free radical/ROS production (Wilson et al 2020). In cerebral (Bujan et al. 2020) and hepatic tissues (Paquot et al 2019), ethanol can boost CYP2E1 mediated free radical production. Alcohol-induced mitochondrial dysfunction also contributes majorly to the generation of ROS in the brain (Reddy et al. 2013). In the present study, decreased mitochondrial electron transport chain (ETC) complex enzyme activities were observed, which lead to mitochondrial dysfunction in turn ROS generation. Besides, membrane-bound NADPH oxidase (NOX) enzymes also generate superoxide ions. NOX enzymes mediated ROS can affect cell physiology by oxidizing the proteins that initially affect the structure and eventually functions of these proteins. In the central nervous system, these enzymes further have critical roles in cellular functions including nerve cell development/differentiation and cellular signaling (Sorce et al. 2009). Altogether, augmented oxidative stress and decreased antioxidant defenses resulted from chronic ethanol administration. It is evident

from this study that chronic alcohol administered rats showed decreased antioxidant status with elevated TBARS and NADPH oxidase enzyme activity. Lipid peroxidation is with phases initiation, propagation, and termination with the generation of free radicals in an amplified way. Moreover, generated ROS further contributes to increased lipid peroxidation which leads to enhanced oxidative stress and damage to membranes and their components in tissues. The brain is a vital organ and controls body functions. Our histopathological results revealed that alcohol consumption caused degenerative and necrotic changes in the brain when compared with that of control rats which showed normal brain architecture with glial tissue. Also, ROS might oxidize the hormone receptors and cause reduced circulating hormone levels. PSE alone administered rats did not show any abnormal changes in these enzyme complex activities compared to other experimental groups indicating safety of the PSE.

Chronic alcohol abuse/use is associated with various behavioural and pathophysiological ailments. The prime adverse effect of ethanol metabolism is the generation of highly reactive by-product acetaldehyde, which crucially initiates the tissue damage through the formation of ROS followed by other free radicals by a change in reduction-oxidation states of liver cells chiefly and also in the brain (perez et al. 2020). The brain is at greater risk of damage arbitrated by ROS because of fewer oxidative defence mechanisms compared to the liver (Hernandez et.al 2016). Alcohol consumption in particular chronic abuse could impair enzymatic and non-enzymatic antioxidant systems that protect cells against ROS damage. Previous studies revealed that chronic alcohol consumption causes oxidative changes in the brain (Agostini et.al 2017). In the central nervous system, aggregation of ROS increases the susceptibility of brain tissue to damage and leads to severe pathophysiologies. The reactive oxygen species with deregulated anti-oxidant system are known to increase brain tissue injury setting off plethora of cellular and molecular events that results in increased permeability in the blood-brain barrier. These cascade of events further lead to activation of matrix metalloproteinases followed by disruption of cell-cell junctions. In the present study, supplementation of PSE to alcohol-fed rats significantly reduced TBARS and NADPH oxidase activity, this might be due to its strong antioxidant capacity, which was evident from our previous studies, where PSE showed strong DPPH, nitric oxide radical scavenging activity (Bulle et al. 2016a).

The central nervous system and endocrine system regulate biochemical reactions in a coordinated way. Hormones are chemical messengers produced and secreted in response to specific physiological and biochemical events by specific endocrine glands. Hormones instigate a cascade of reactions within the body including alterations in cell and organelle membrane integrity and enzyme/hormone synthesis and secretion may lead to change in behavior of cell type (Rachdoui et al. 2013). In the present study, significantly decreased circulating levels of plasma T_3, T_4, TSH, estradiol, testosterone in alcohol administered rats were observed. Moreover, the concentration of the steroidal hormone, cortisol levels in the plasma of alcoholic rats was significantly elevated. The hypothalamus in coordination with the anterior pituitary gland and the other target endocrine glands such as adrenal, thyroid, and gonads forms the axes (HPA/HPT/HPG) and thereby regulates several specific functions. The majority of studies revealed the direct effect of alcohol on HPA axis activity (Rasmussen et al. 2000, Ogilvie et al. 1998). Alcoholism and alcohol abuse is linked to HPA activity and homeostasis resulting in the alterations of

cortisol and corticotropin-releasing factor which intern induce a variety of pathological conditions (Blaine et al. 2017). Endocrine system maintains cellular homeostasis and controls metabolism, energy levels, electrolyte balance, growth, development, and reproduction. Cellular macromolecules like proteins and enzymes are inactivated by alcohol-induced ROS by increasing lipid peroxidation, oxidative stress, and further infers with physiological processes which enhance cellular toxicity. Oxidative stress impairs glucocorticoid receptor function (Tian et al. 2019, Schiavone et al. 2013). Increased hydroxyl radical production is responsible for impaired nuclear translocation of the glucocorticoid receptor and its dysfunction (Marty et al. 2020, Tanaka et al.1999). A disturbance in the HPG axis can result in an imbalance in plasma circulating hormone levels. Several mechanisms may be involved in the alcohol-induced changes in the circulating levels of hormones. One of the known mechanisms of action is alcohol's ability to increase oxidative stress. Although the exact mechanisms between oxidative stress and the endocrine status are unknown. However, ROS may affect the endocrine status *via* several molecular mechanisms: i. Alcohol consumption increases brain oxidative stress and alters mitochondrial complex activities, leading to neurological disorders; ii. Chronic alcohol consumption also modulates kinases and cysteine-rich, redox-sensitive proteins which alter transcription and RNA stability leading to reactive oxygen species synthesis by NOX2 and mitochondrial ETC complexes; iii. ROS produced by the mitochondrial ETC complex and CYP-450 causes the release of intracellular calcium, which in turn phosphorylates PKC and PLC to form an active NOX complex; iv. Alcohol induces inflammatory pathways that activate NOX-mediated reactive oxygen species production and the expression of cytokines and iNOS (inducible nitric oxide synthase).

Previous studies also revealed that resveratrol, one of the potential active principles from *P. santalinus* alleviates ethanol-induced hormonal and metabolic disturbances (Szkudelska et al. 2017). Our studies revealed that there is a direct relationship between alcohol-induced oxidative stress and altered hormonal levels. However, supplementation of PSE to alcohol-fed rats prevented these hormonal alterations by scavenging free radicals as well as its metal chelating ability. The bioactive constituents in PSE actively might have played a role in this mitigation effect.

Conclusions

Hormonal receptors and NADPH oxidase are localized in the membrane. Thus, ROS produced by mitochondria and NADPH oxidase *via* PLC and PKC dependent pathway alters hormonal receptors and lead to alterations in the synthesis and physiological functioning of the hormones. The phytocompounds present in PSE modulate the altered hormonal status by scavenging ROS or decreasing oxidative stress and thereby offers protection against alcohol-induced neurotoxicity.

Declarations

Author’s contribution

VDR, SM, ZB designed work, SB, SP, PP performed experiments, KB, VDR SA and NCh wrote paper. All authors read and approved the final manuscript.

Funding information

Dr. Bulle Saradamma and Dr.V.Damodara Reddy thank SERB, New Delhi, India for providing financial support under N-PDF (PDF/2017/001313 dated 06-10-2017) and Ramanujan Fellowship schemes (SB/S2/RJN-043/2014 dated 17.12.2015) respectively.

Availability of data and material

The datasets generated during and/or analysed in the current study are obtainable from the corresponding author on request.

Code availability: Not applicable

Ethics approval: The present study was conducted upon approval of Sri Krishnadevaraya University institutional animal ethics committee (471/06/c/CPCSEA, dt.20th Sep 2016).

Consent to participate: Not applicable

Consent for publication: Not applicable

Conflicts of Interest

All authors declare that there is no conflict of interest

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Tables

Table 1. Effect of PSE on brain antioxidant enzymes and glutathione content in alcohol administered rats.

Parameter	Control	Alcohol	PSE	Alcohol+PSE
GSH (µg/mg protein)	87.3 ± 7.3	51.7 ± 4.2*	90.6 ± 6.4	79.4 ± 8.2^{ns}
GPx (µmol/min/mg protein)	13.6 ± 1.4	7.9 ± 1.8*	12.3 ± 2.33	12.1 ± 1.9^{ns}
GR (µmol/min/mg protein)	18.4 ± 2.6	7.8 ± 1.3*	16.9 ± 2.4	17.1 ± 3.5^{ns}
GST (µmol/min/mg protein)	9.2 ± 1.4	4.9 ± 1.2*	6.8 ± 2.3	8.3 ± 2.2^{ns}
SOD (units/min/mg protein)	22.8 ± 3.2	14.1 ± 2.7*	21.6 ± 3.1	20.7 ± 3.6^{ns}
CAT (µmol/min/mg protein)	3.2 ± 0.25	1.6 ± 0.09*	3.5 ± 0.05	2.9 ± 0.08^{ns}

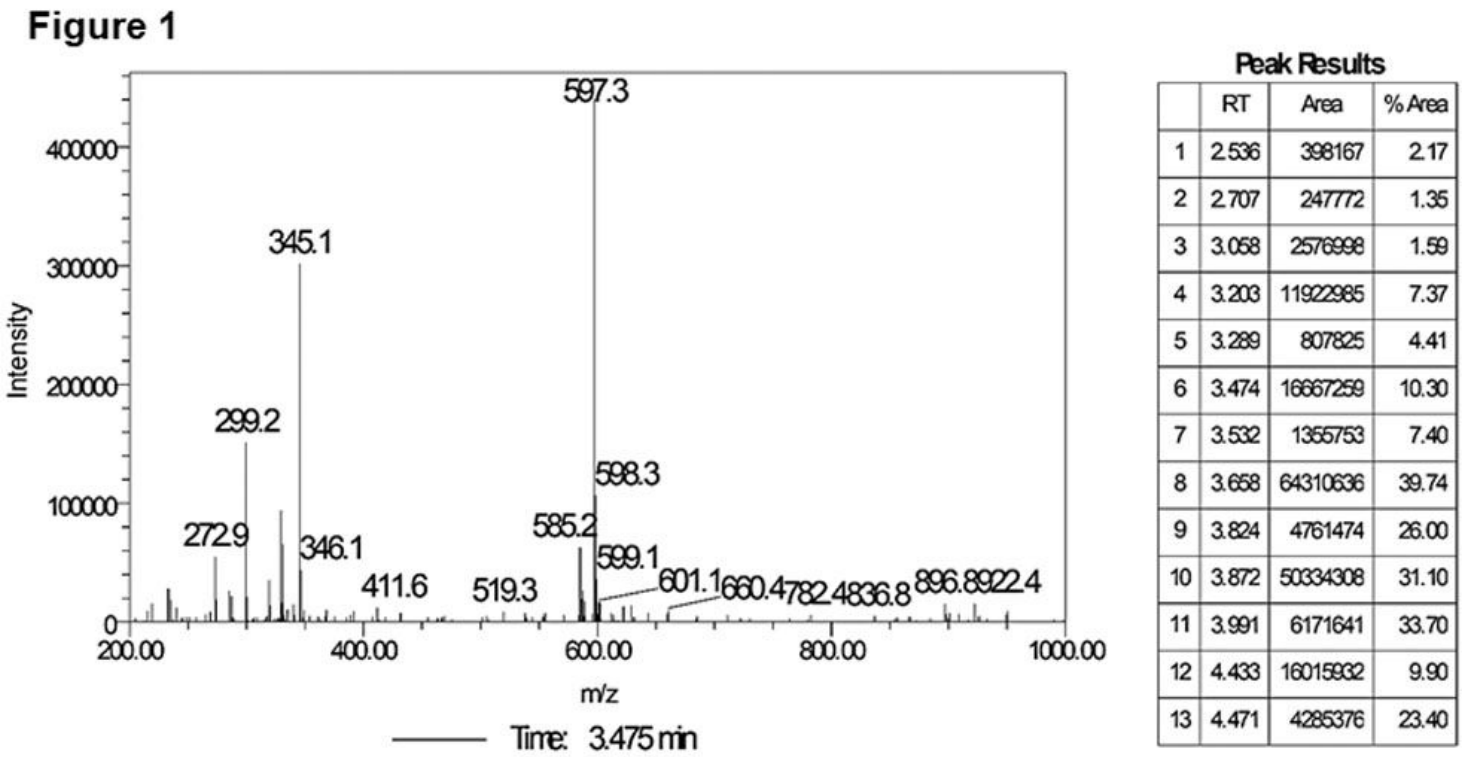
GSH is expressed in µg/mg protein and remaining values as µmole/min/mg ptn. Values presented as the mean ± SD. A $p < 0.05$ is considered as significantly different between group. "*" indicates significant from control, "ns" indicates not significant from controls and PSE alone administered rats.

Table 2. Effect of PSE on plasma endocrine status in alcohol administered rats.

Parameter	Control	Alcohol	PSE	Alcohol+ PSE
T_3(ng/dl)	168 ± 16.3	125 ± 9.1*	155 ± 13.2	161 ± 11.9ns
T_4 (µg/dl)	25.9 ± 2.3	15.2 ± 1.9*	22.3 ± 2.6	26.7±1.4ns
TSH (MIU)	2.8 ± 0.2	1.04 ± 0.08*	2.4 ± 0.65	2.6 ± 0.2ns
Testosterone (ng/ml)	22.9 ± 2.4	12.7 ± 1.5*	20.5 ± 1.9	21.4 ± 2.4ns
Estradiol (pg/ml)	181 ± 14.3	154 ± 12.8*	173 ± 14.7	175 ± 13.2ns
Cortisol (µg/dl)	209 ± 11.5	250 ± 16.2*	224 ± 24.1	211 ± 18.8ns

Values are represented as the mean ± SD. A $p < 0.05$ is considered as significantly different between groups. “*” indicates significant difference from controls, “ns” indicates not significant from controls and PSE alone administered rats.

Figures



	RT	Area	% Area
1	2.536	398167	2.17
2	2.707	247772	1.35
3	3.058	2576998	1.59
4	3.203	11922985	7.37
5	3.289	807825	4.41
6	3.474	16667259	10.30
7	3.532	1355753	7.40
8	3.658	64310636	39.74
9	3.824	4761474	26.00
10	3.872	50334308	31.10
11	3.991	6171641	33.70
12	4.433	16015932	9.90
13	4.471	4285376	23.40

Figure 1

Liquid chromatography-mass spectroscopic (LC-MS) analysis of aqueous extract of Pterocarpus santalinus heartwood (PSE).

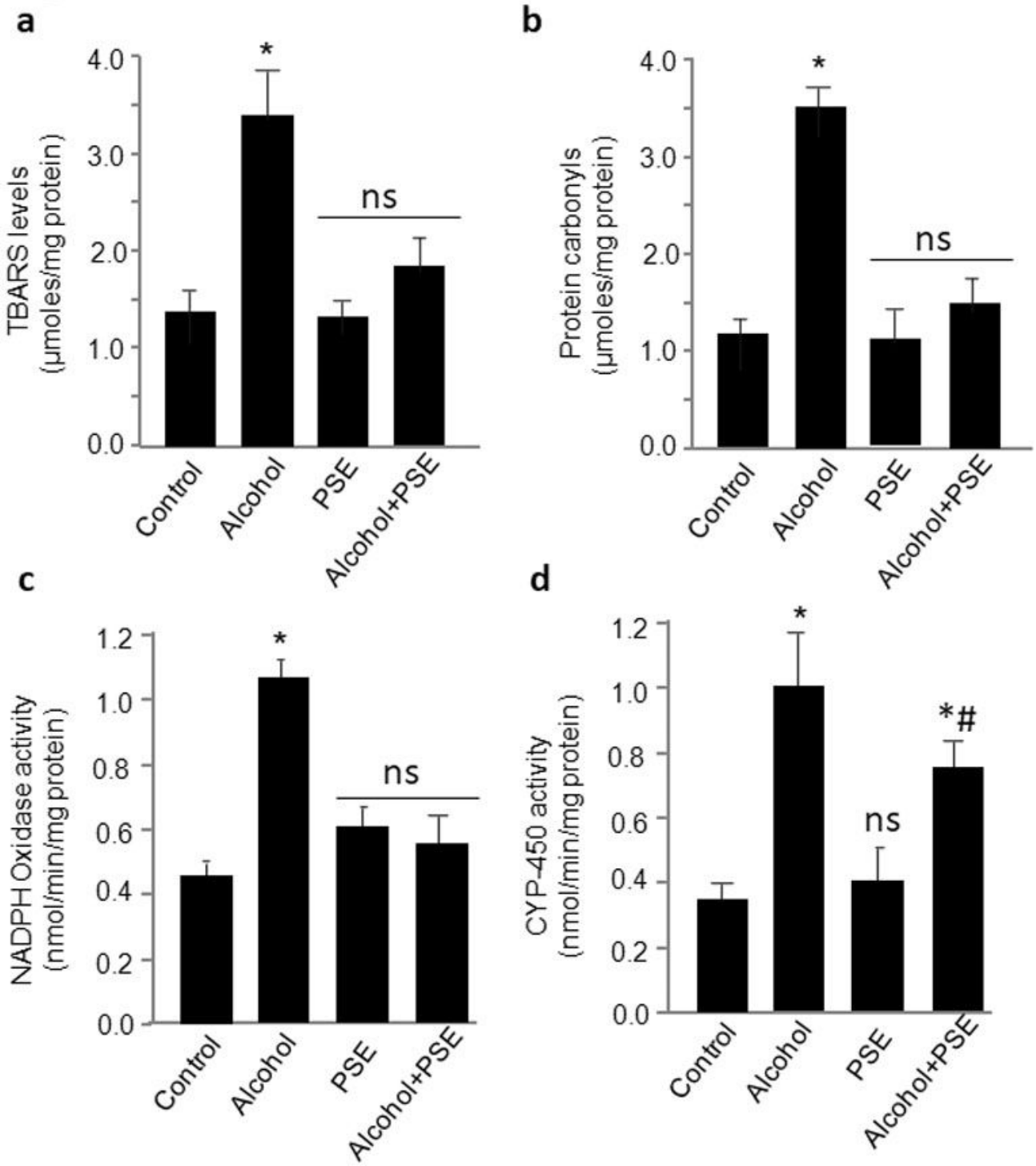


Figure 2

Effect of PSE on ethanol fed experimental rats. (a) TBARS, (b) protein carbonyls, (c) NADPH oxidase activity, (d) CYP-450 enzyme activity in alcohol administered rats. Values represent mean ± standard deviation (n=8). A $p<0.01$ represents significance between different groups. "*" indicates significant difference compared to controls, "#" indicates significant difference compared to controls and alcohol administered rats, "ns" indicates no significance from controls and PSE alone administered rats.

Figure 3

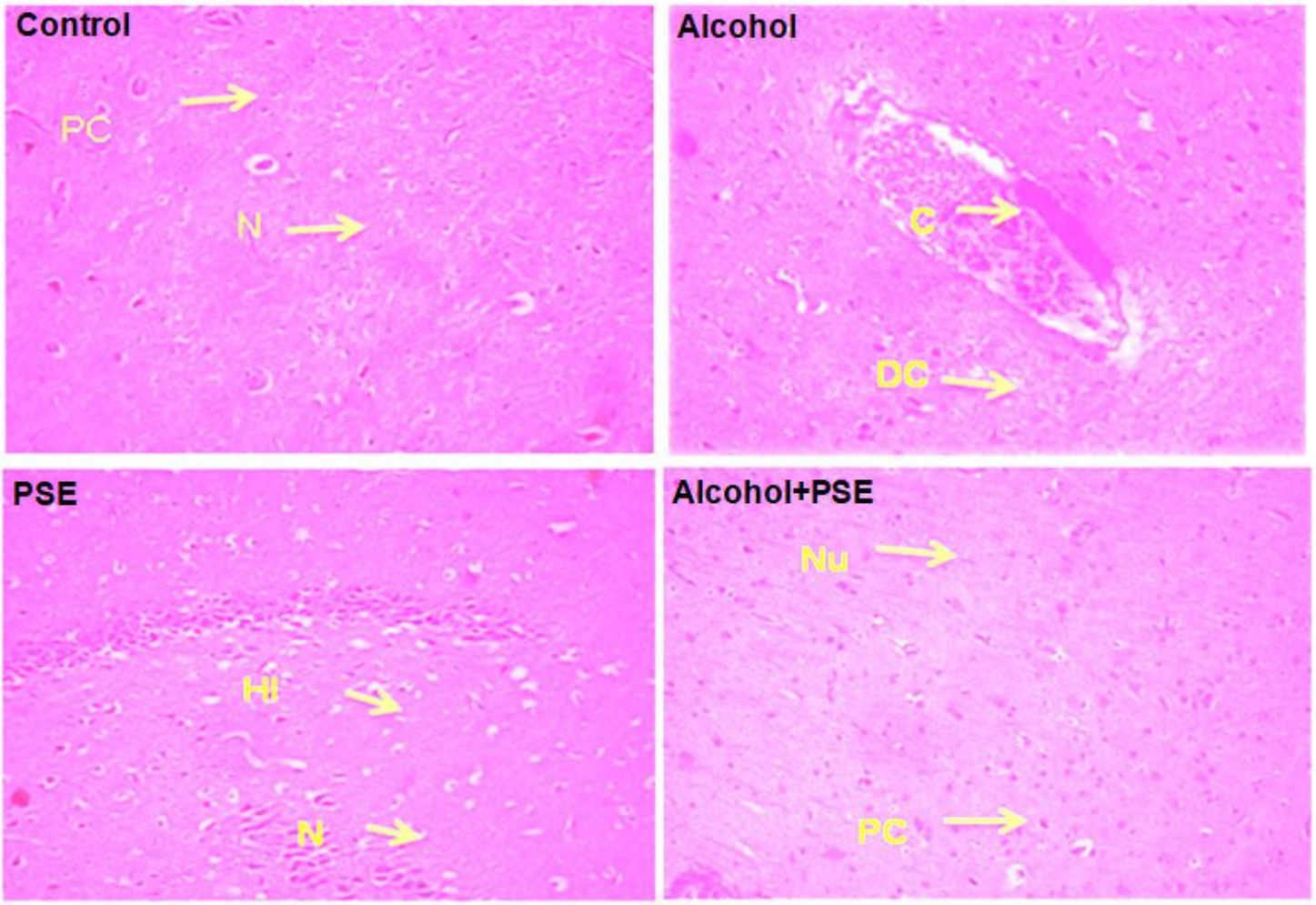


Figure 3

Representative photomicrographs of the brain in different alcohol ingested experimental rat groups. a. Brain sections from control rats showed no signs of pathological signs. b. Brain morphology of alcohol administered rats clearly showed degenerative and necrotic changes, whereas c. PSE alone administered rat brain sections. d. alcohol and PSE administered rats showed normal brain architecture with glial tissue (magnification, 100 X).

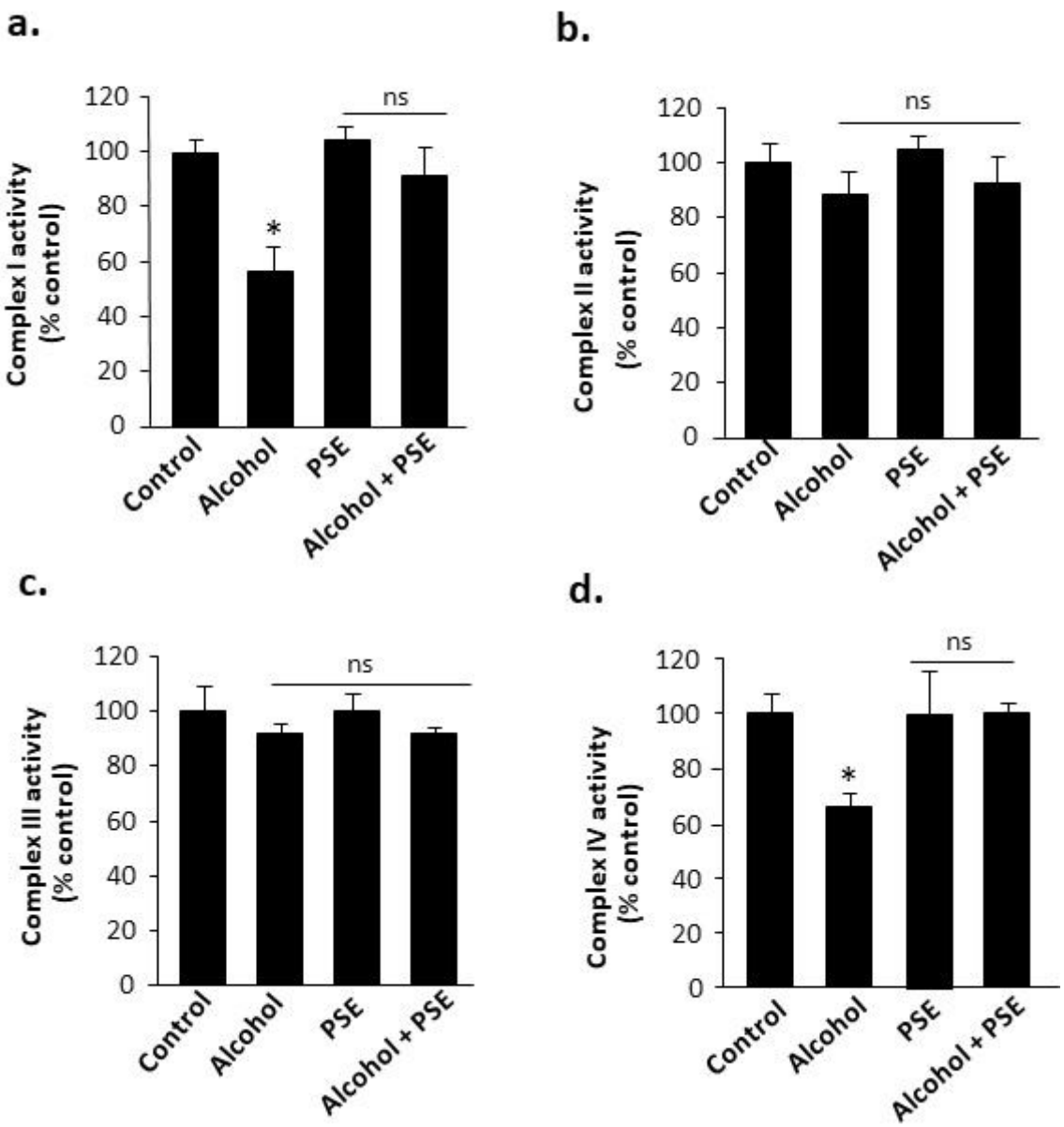


Figure 4

Alcohol induced alterations in mitochondrial complex activities/functions. (a) Complex-I activity (b) Complex-II activity (c) Complex-III activity and (d) Complex-IV activity. Data was presented as mean ± standard deviation and expressed as the percentage of the control. The control values of Complex-I, -II, -III and -IV are 2657 ± 53, 177 ± 13, 1214 ± 61, and 1848 ± 36 respectively. “*” indicates statistical significance compared to control rats. *p<0.05; n.s., not significant.

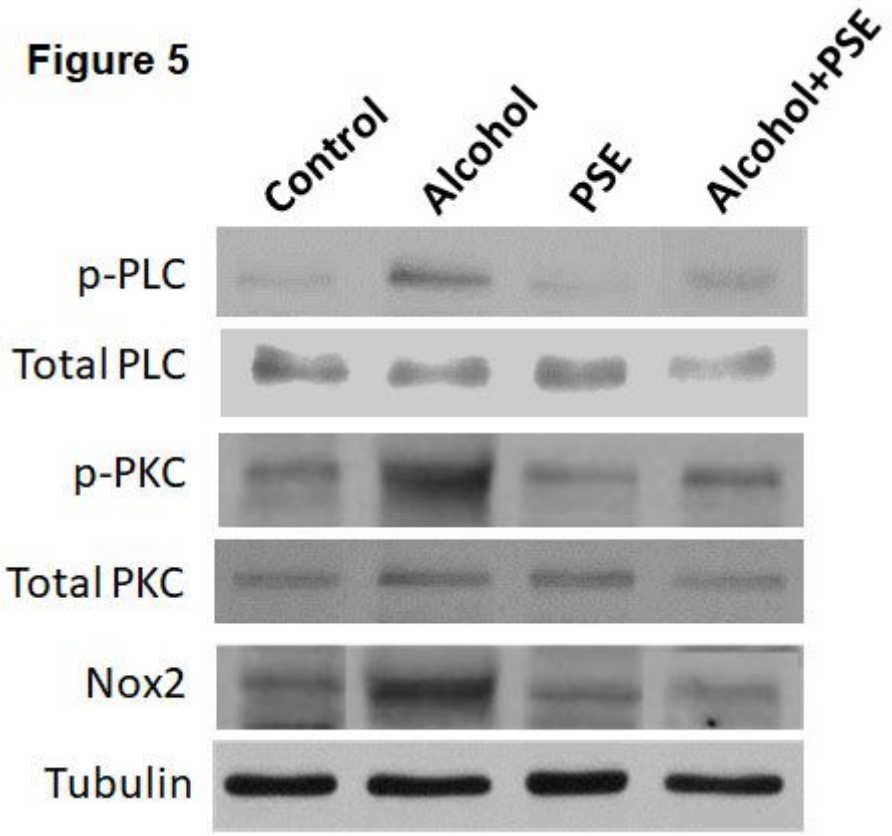


Figure 5

Effect of PSE administration on phosphorylated PLC, total PLC, phosphorylated PKC, total PKC, NOX2, and α-tubulin protein expression (loading control) in control and alcohol-treated rats. The results are expressed as mean ± standard deviation of three independent experiments.