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ATTENUATION OF EXPERIMENTAL PULMONARY HYPERTENSION BY GRAPE SEED EXTRACT AND PUNARNAVA EXTRACT

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ABSTRACT

Ethnopharmacological relevance: Proliferation of pulmonary arterial smooth muscle cells, endothelial dysfunction, oxidative stress and inflammation promotes the development of pulmonary hypertension. Grape seed extract (GSE) is processed from the seeds of the grapes *Vitis Vinifera L*. It has shown to have antioxidant and vasodilating effects, but its effects on pulmonary arteries remain poorly defined. Punarnava extract is processed from the herb *Boerhaavia Diffusa*. It has been well reported that extract of Boerhaavia Diffusa, a plant used in Indian traditional medicine, has significant immunomodulatory potential. The present study was undertaken to investigate the efficacy of GSE and PE to attenuate pulmonary hypertension. ***Material and method:*** Rats injected with monocrotaline (60mg/kg., s.c, once in 4 weeks) progressively developed pulmonary hypertension. Pulmonary hypertension was assessed in terms of attenuation of acetylcholine-induced endothelium-dependent relaxation; Sodium nitroprusside induced endothelium independent vasorelaxation (Isolated pulmonary artery ring preparation), decrease in serum nitrate/nitrite level, and increased oxidative stress (SO^{2-} and TBARS level), right ventricular systolic pressure and right ventricular hypertrophy. Pulmonary artery medial hypertrophy was assessed using H and E staininig. ***Results:*** Treatment with GSE and PE attenuated right ventricular systolic pressure, right ventricular hypertrophy, pulmonary artery medial hypertrophy, decreased oxidative stress and increases

acetylcholine induced endothelium dependent relaxation, serum nitrate/nitrite level and mRNA expression of eNOS. ***Conclusion:*** Thus it may be concluded that GSE and PE ameliorates pulmonary vascular endothelium dysfunction and pulmonary hypertension possibly by increasing the eNOS activity and expression.

KEYWORDS: Pulmonary Vascular endothelium dysfunction, endothelial nitric oxide synthatase (eNOS), Nitric oxide.

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is defined by a mean pulmonary artery pressure of >25 mmHg (Montani et al., 2013) and a normal pulmonary artery wedge pressure (PCWP) of <15 mmHg which is characterized by abnormally thickened pulmonary arteries, right ventricular hypertrophy and ultimately premature death (Schermuly et al., 2011; Mathew et al., 2009). PAH is caused by the imbalance between the normal level of vasodilators and vasoconstrictors, growth inhibitors and mitogenic factors and antithrombotic and prothrombotic determinants. These include prostacyclin and thromboxane $A_{2,}$ endothelin-1, nitric oxide, serotonin, adrenomedullin, vasoactive intestinal peptide, hypoxia, anorexigens. There are several other coexisting conditions having mechanistic links with pulmonary arterial hypertension associated with scleroderma, and infection with the human immunodeficiency virus (HIV), human herpesvirus (HHV), portal hypertension, thrombocytosis, hemoglobinopathies, and hereditary hemorrhagic telangiectasia (Harrison et al., 2004).

Current treatment for PH consists of mostly the vasodilators such as phosphodiestrase inhibitors, ET1 antagonist, prostacyclin analogue etc. that have effect on vascular tone , However Cardiac abnormalities as a result of Pulmonary hypertension remains untreated. Various herbal extract are available that have multiple effects on the lungs, blood vessels and the heart. GSE and PE are two of the extensively reported extracts that have shown beneficial effects on cardiovascular system. GSE have been shown to activate endothelial nitric oxide synthase (eNOS), and cause an endothelium-dependent relaxation (EDR) of blood vessels. It has also been shown that grapes seed extract increase the antioxidant levels and improve the endothelial function (Sivaprakasapillai et al., 2009). It has been reported that punarnava extract, has significant immunomodulatory potential (Rajpoot et al., 2011). To test this hypothesis we investigated the potential role of GSE and PE in monocrotaline-treated rats. Rats develop severe pulmonary hypertension after a single injection of monocrotaline (MCT),

(Rosenberg et al., 1988) and this model mimic several key aspects of pulmonary hypertension, including vascular remodeling, proliferation of PASMCs, oxidative stress and endothelial dysfunction. Thus the present study is designed to investigate the potential effect of grape seed extract (vasoprotective) and punarnava extract (immunomodulator) in monocrotaline induced pulmonary vascular endothelium dysfunction.

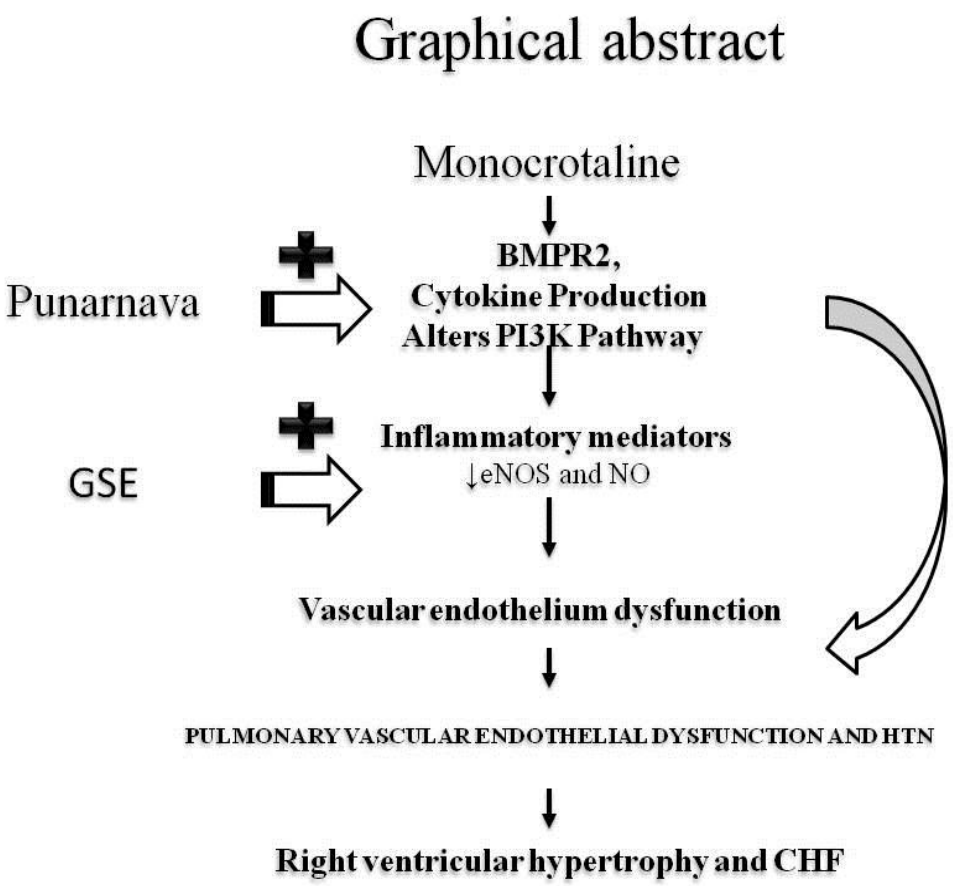


Fig. no. 1

2. MATERIALS AND METHODS

2.1 Experimental Animals

The experimental protocol used in the present study was conducted in approval with Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the guidelines of the Guide for the care and use for laboratory animals. Wistar rats (220-300 gm) were used of either sex.

2.2 Drugs and Chemicals

Bosentan was purchased from Cipla ltd, Monocrotaline from Sigma Aldrich, Grape seed extract was obtained as a gift sample from Biogen extract Pvt. Ltd. and punarnava extract

was obtained as a gift sample from Hindustan Mint and Agro products Pvt. Ltd. All the other chemicals and biochemical reagents used were of analytical grade.

2.3 Confirmatory test for L-Arginine in grape seed extract (Sakaguchi test)

To 1 mL of grape seed extract solution in a test tube, add 40%NaOH (2-4 drop) + Ethanolic a-Naphthol (2 drops) and Bromine water (5-10 drops) − Mix well; a red-colour complex will be formed with Arginine.

2.4 In-vitro antioxidant studies of different ratio of the grape seed extract and punarnava extract: DPPH method (Elizabeth et al., 1990)

Different concentration of GSE and PE were prepared in methanol at concentrations 50, 100, 150, 200 and 250 µg/ml. The antioxidant activity of extracts was carried out using two different methods: free radical scavenging activity using DPPH.

2.4.1 DPPH radical-scavenging activity

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts. Different concentrations of each extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard controls. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Table No. 1

	Experimental protocol	**(Each group n=6)**
SNo.	**Groups**	**Treatment**
1.	Group I (Normal control)	Rats were maintained on normal chow diet with drinking water
2.	Group II (Monocrotaline control**)**	MCT (60mg/kg;s.c) was administered once in four weeks
3.	Group III (MCT + GSE)	MCT (60mg/kg; s.c) administered rats were treated with Grape seed extract (150mg/kg/day; p.o) from 21st to 28th day.
4.	Group IV (MCT + GSE)	MCT (60mg/kg/day;s.c) administered rats were treated with Grape seed extract (300mg/kg/day; p.o) from 21st to 28th day.
5.	Group V (MCT+PE)	Monocrotaline (60mg/kg/day;s.c) administered rats were treated with Punarnava extract (100mg/kg/day; p.o) from 21st to 28th day
6.	Group VI (MCT+PE)	Monocrotaline (60mg/kg/day;s.c) administered rats were treated with Punarnava extract (200mg/kg/day; p.o) from 21st to 28th day
7.	Group VII (MCT+GSE+PE)	Monocrotaline (60mg/kg/day;s.c) administered rats were treated with Grape seed extract (150mg/kg/day; p.o) and Punarnava extract (100mg/kg/day; p.o) from 21st to 28th day
8.	Group VIII (MCT+ Bosentan)	Monocrotaline (60mg/kg/day;s.c) administered rats were treated with bosentan (200mg/kg) from 21st to 28th day

2.5. Assessment of pulmonary artery vascular endothelium dysfunction

2.5.1. Assessment of right ventricular hemodynamics

The animals were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal). A polyethylene catheter was inserted into the right ventricle (RV) through the jugular vein for the measurement of RV systolic pressure (RVSP). Hemodynamic variables were measured using a physiograph volume transducer (T-301 transducer) (Csiszar et al., 2009).

2.5.2. Endothelial function of pulmonary arteries by using wire myograph

The rats were sacrificed by cervical dislocation, followed by decapitation. Pulmonary artery was removed, cut into a ring of 4-5 mm in length and mounted in a 5ml organ bath of wire myograph (Radnoti, US) containing krebs-Henseleit solution and maintained at 37°C (Kuwano et al., 2008). The preparation was allowed to equilibrate for 90 min under 1.5 g tension. The isometric contractions were recorded on the computer with the help of data acquisition system (Power lab 4/30 Ad Instrument). The pulmonary artery ring preparation was primed with 80mM KCl to check its functional integrity and to improve its contractility. The cumulative dose responses of ACh (10^{-8} to 10^{-4} M) were recorded in phenylephrine (3×10^{-6} M) precontracted preparation. Furthermore sodium nitroprusside (10^{-8} to 10^{-4} M) induced endothelium independent vasorelaxation was assessed in isolated pulmonary artery ring preparation with intact or denuded endothelium (denuded using tungsten wire of 75 µm, Radnoti USA) respectively (Shah and Singh, 2007; Csiszar et al., 2009).

2.5.3. Assessment of mRNA expression of eNOS

100 mg of pulmonary artery tissue was homogenized in 1ml of trizol reagent and incubated for 5 minute at 20°C. 0.2ml of Chloroform was added to homogenate, incubated at 20°C for 3 minutes. Mixture was centrifuged at 10,000 g for 15 minutes at 4°C, upper aqueous phase was isolated and 0.5ml isopropyl alcohol was added to precipitate RNA (For Isolation of RNA XNAPS RNA Flexspin Kit, Renogen). The sample was centrifuged at 10,000g for 15 minutes at 4°C to form gel like pellet of RNA in the tube. The supernatant was removed , RNA pellet was washed with 75% ethanol, mixed , centrifuged at 7500g for 5 min at 4°C and RNA pellets were briefly vacumm dried for 5-10 min. The RNA was quantified by ultraviolet absorbance spectrophotometrically to ascertain A260/A280 ratio < 1.6 and dissolved in RNAase free water (rtPCR Kit, Renogen) 5ul reverse primer was added to crude RNA, 29ul reverse transcriptase buffer incubated for 10 min at 65°C and collected on ice. 16U AMU transcriptase (10U/ul) and 5 ul 10Mm dNTP mixture were added, incubated at 42°C for 1

hour and 10mM Tris buffer (pH 7.5) was added to synthesize single stranded cDNA. 5ul of cDNA product was mixed with 4ul dNTP mixture, 5ul forward primer, backward primer, 10X amplification buffer, 0.9ul of Taq DNA polymerase enzyme (3U/ul) and 70.1ul RNase free water in PCR tube and overlaid with 100ul mineral oil. Twenty-four PCR cycles of GAPDH (94°C for 1 minute, 62°C for 1 minute, 72°C for 1 minute) and thirty PCR cycles of eNOS (94°C for 1 min, 62°C for 1 minute, 72°C for 1 minute) followed by 1 cycle at 57 °C for 2 min and 72°C for 7 minute were performed by using one half of the reverse transcription mixture (Biorad, MJ Mini Thermal cycler). The sense and antisense **primers of eNOS**: (5'-TCCAGAAACACAGACAGTGCA-3' and 5' CAGGAAGTAAGTGAGAGC-3' respectively), and **primers of GAPDH** (5'-TCCCTCAAGATTGTCAGCAA-3'and 5' AGATCCACAACGGATACATT-3' respectively) were used. The PCR products so obtained were analyzed on ethidium-bromide stained agarose (1.5%) gel on Gel Electrophoresis apparatus (Biorad). The eNOS and GAPDH products were quantified using image J (Gel Doc EZ image, Biorad) and amount of eNOS was normalized with respect to amount of GAPDH product (Shah and Singh., 2007).

2.5.4. Estimation of serum nitrite/nitrate concentration

A total of 400ul of carbonate buffer (pH 9.0) was added to 100ul of serum sample followed by the addition of small amount (0.15 g) of copper–cadmium alloy. The tubes were incubated at room temperature for 1 h to reduce nitrate to nitrite. The reaction was stopped by adding 100 ul of 0.35 M sodium hydroxide. Following this, 400 ul of zinc sulfate solution (120 mM) was added to deproteinate the serum samples. The samples were allowed to stand for 10 min. and then centrifuged at 4,000g for 10 min. Greiss reagent (250 ul of 1.0% sulfanilamide and 250 ul of 0.1%N-naphthylethylenediamine) was added to aliquots (500 ul) of clear supernatant and serum nitrite/nitrate was measured spectrophotometrically at 545 nm. The standard curve of sodium nitrite (1–40uM) was plotted to calculate concentration of serum nitrite (Jindal et al., 2008).

2.6. Assessment of oxidative stress

2.6.1. Serum thiobarbituric acid reactive substances (TBARS)

A total of 1 ml of 20% trichloroacetic acid was added to 100 µl of serum and 1% thiobarbituric acid (TBA) reagent (1.0 ml), which were mixed and incubated at 100°C for 30 min. After cooling on ice, samples were centrifuged at 1,000g for 20 min. Serum concentration of TBARS was measured spectrophotometrically at 532 nm. A standard curve

using 1, 1, 3, 3-tertramethoxyopropane (1–10 uM) was plotted to calculate the concentration of TBARS (Jindal et al., 2008).

2.6.2. Estimation of superoxide anion

Pulmonary artery was cut into transverse rings of 6 mm in length and placed in 5 ml of Krebs–Henseleit solution containing 100uM of nitroblutetrazolium (NBT) and incubated at 37°C for 1.5 h. The NBT reduction was stopped by adding 5 ml of 0.5 N HCl. The rings were minced and homogenized in a mixture of 0.1 N NaOH and 0.1% sodium dodecoylsulphate (SDS) in water containing 40 mg/l of diethylenetriaminepentaacetic acid (DTPA). The mixture was centrifuged at 20,000g for 20 min and the resultant pellets were resuspended in 1.5 ml of pyridine and kept at 80°C for 1.5 h to extract formazan. The mixture was centrifuged at 10,000g for 10 min and the absorbance of formazan was determined spectrophotometrically at 540 nm. The amount of reduced NBT was calculated using the following formula.

$$Amount\ of\ reduced\ NBT = \frac{\mathrm{A\,x\,V}}{\mathrm{T\,x\,Wt\,x\,e\,x\,l}}$$

Where A is absorbance, V is volume of solution (1.5 ml), T is time for which pulmonary artery rings were incubated with NBT (90 min), Wt is blotted wet weight of pulmonary artery rings, e is extinction coefficient (0.72 l $mM^{-1}mm^{-1}$), and l is length of light path (10 mm). The result was expressed as reduced NBT in picomoles per min per mg of wet tissue (Jindal et al., 2008).

2.7. Measurement of Right ventricular Hypertrophy

The heart was dissected, and the ratio of the right ventricular free wall weight divided by the length of the tibia was calculated as an index of right ventricular hypertrophy which is unaffected by changes in body weight or left ventricular mass (Cowan et al., 2000).

The ratio was calculated by using formula

$$Ratio = \frac{\text{Right ventricular}}{\text{Left ventricular} + \text{Septum}}$$

2.8. Histological studies

The specimens were preserved in phosphate-buffered 10% formalin. Lung tissues were washed, distended appropriately and dissected into small pieces. Subsequently, tissues were embedded in paraffin and cut into 5 µm slices. The sections were stained with haematoxylin-

eosin and pulmonary vascular wall thickness was assessed under light microscopy ((Kolettis et al., 2007).

3. Statistical Analysis

All values are expressed as mean ± S.D. Data for isolated pulmonary artery ring preparation was statistically analyzed using one way ANOVA followed by Newman Keuls test. The data for right ventricular systolic pressure, nitrite, superoxide anions, TBARS and RVH were statistically analyzed using one way ANOVA followed by Tukey's multiple comparison tests. P value ≤ 0.05 was considered to be statistically significant.

4. RESULTS

4.1. Confirmatory test for L-Arginine in Grape seed extract: GSE with NaOH (2-4 drop) + Ethanolic a-Naphthol (2 drops) and Bromine water (5-10 drops) formed red colour complex. It shows the presence of L- arginine in GSE.

4.2. Antioxidant assays

The antioxidant activity of the GSE and PE was measured on the basis of its DPPH and superoxide radical scavenging activity. The concentration of the extracts needed for 50% scavenging (IC50) of DPPH and superoxide was found to be 63.98µg/ml and 67.83µg/ml respectively. Ascorbic acid was used as positive control for which the IC50 value was found to be 42.28µg/ml respectively. The results were shown in Table.

Table No. 2.

S. No	Samples	IC_{50} Value (µg/ ml)
1	Ascorbic acid	42.28 ± 0.5
2	Extract of grape seed	63.98 ± 1.9
3	Extract of punarnava	67.83 ± 2.5

5. Effect of Pharmacological interventions on pulmonary hypertension

5.1. Effect of Pharmacological interventions on right ventricular systolic pressure

MCT administered rat consistently developed significant pulmonary hypertension. Consequently, RV systolic pressure was increased significantly as compared with the normal control. However, treatment with GSE (150 and 300 mg/kg; p.o), PE (100mg/kg and 200 mg/kg, p.o) alone and in low dose combination and bosentan (200 mg/kg; p.o) in normalize RV systolic pressure in MCT-injected rats (Fig. 2).

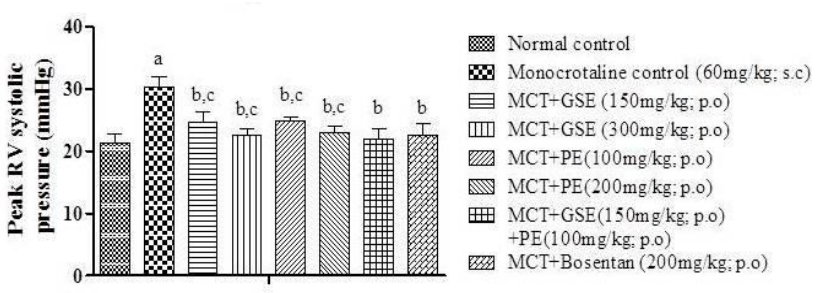


Fig. 2.

5.2. Effect of pharmacological interventions on endothelium dependent and independent relaxation

Acetylcholine (Ach) (Fig.3) and sodium nitroprusside (SNP) (Fig.4) causes endothelium dependent and independent relaxation, respectively in phenylephrine (3 X 10^{-6} M) precontracted isolated normal rat pulmonary artery ring preparation in a dose dependent manner. In MCT administered rats ach induced endothelium dependent relaxation was noted to be significantly attenuated grape seed extract (150 and 300 mg/kg; p.o) and punarnava extract (100 and 200 mg/kg; p.o) alone and in low dose combination and bosentan (200 mg/kg; p.o), significantly improved ach-induced endothelium dependent relaxation in pulmonary artery rings. However SNP induced endothelium independent relaxation remained unchanged.

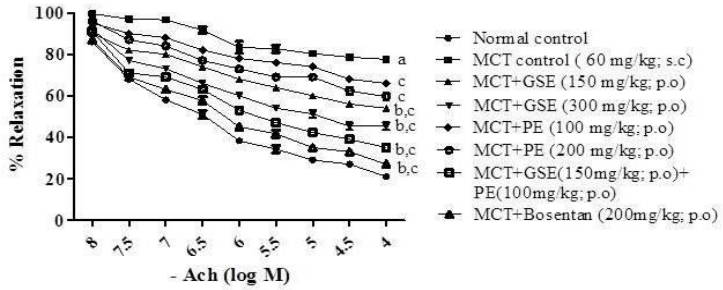


Fig.3

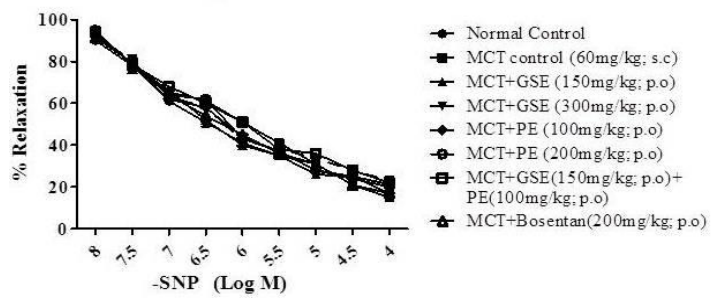


Fig.4

5.3. Effect of pharmacological interventions on mRNA level of eNOS

Monocrotaline administration decreases mRNA level of eNOS as compared to the normal control group. However treatment with GSE (150 and 300 mg/kg; p.o), PE (100mg/kg and 200 mg/kg, p.o) alone and in low dose combination and bosentan (200 mg/kg; p.o) markedly increases mRNA level of eNOS in MCT administered groups (Fig. 5).

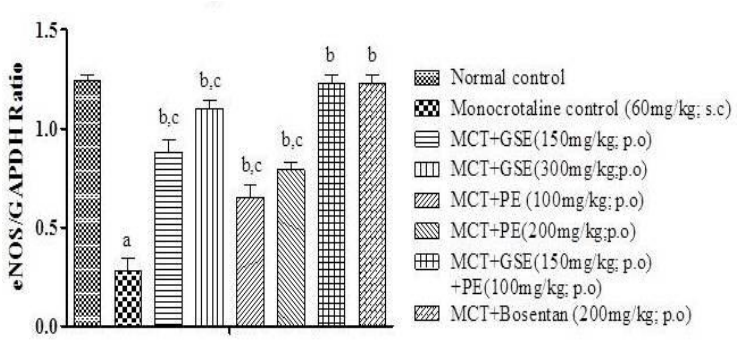


Fig. 5

5.4. Effect of Pharmacological interventions on serum nitrite/nitrate concentration

The serum nitrite/nitrate concentrations were noted to be reduced in MCT administered groups when compared with normal control. However treatment with GSE (150 and 300 mg/kg; p.o), PE (100 and 200 mg/kg, p.o) alone and in low dose combination significantly increases serum nitrite/nitrate concentrations. Moreover bosentan (200 mg/kg; p.o) markedly restored the reduced concentration in MCT administered groups (Fig.6).

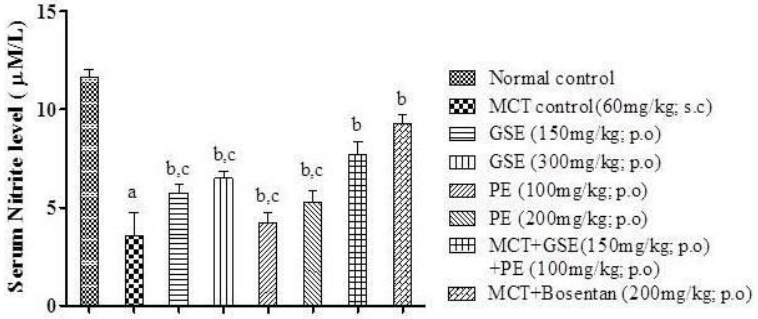


Fig.6

5.5. Effect of Pharmacological interventions on pulmonary artery superoxide anion generation, serum TBARS and right ventricular hypertrophy

Pulmonary artery superoxide anion and serum TBARS was significantly increased in monocrotaline control groups in comparison to normal control group. However treatment with GSE (150 and 300 mg/kg; p.o) and PE (200 mg/kg; p.o) alone and in low dose

combination and bosentan (200 mg/kg; p.o) significantly decreases superoxide anion level, TBARS level and has shown significant reduction in RV (LV+septum) ratio in comparison to MCT control groups. (Fig.7, Fig.8 and Fig.9).

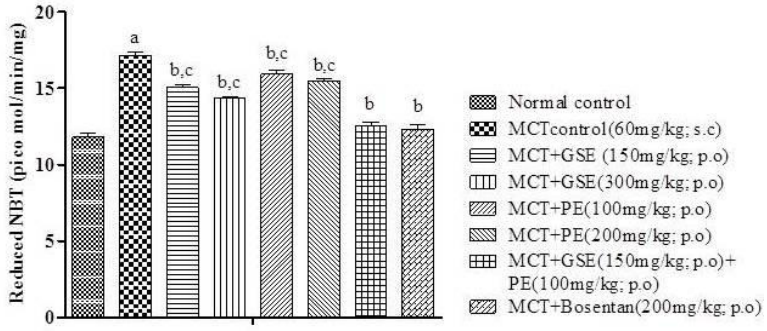


Fig.7

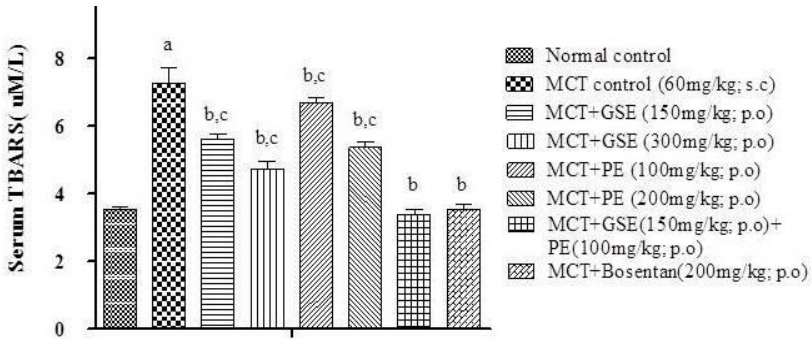


Fig.8

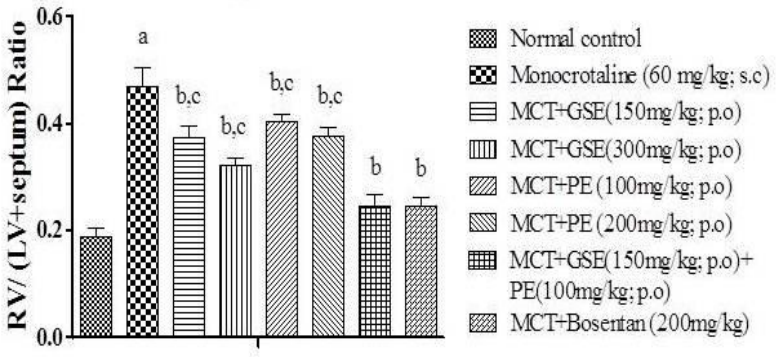
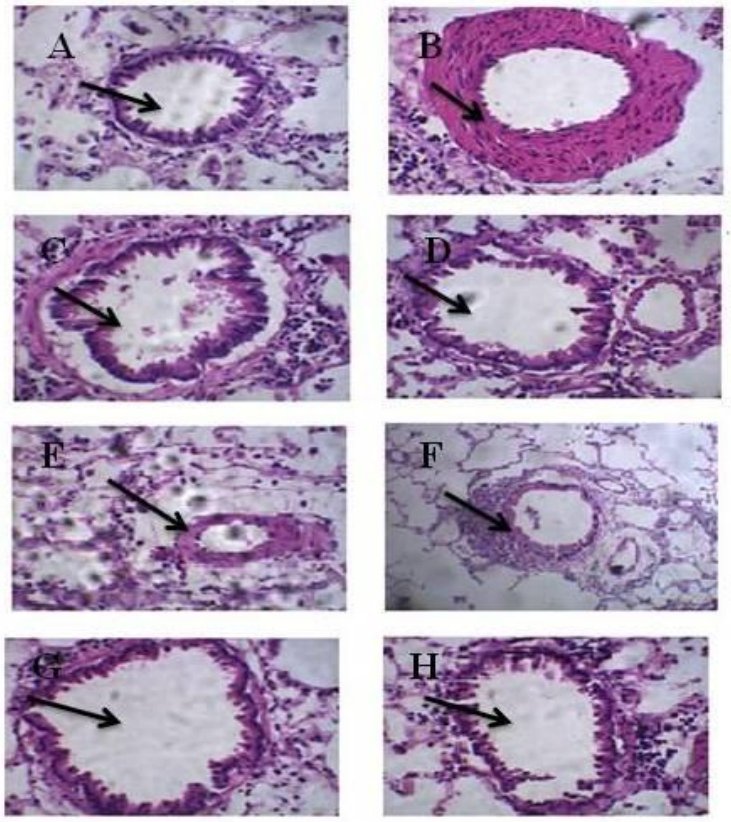


Fig.9

5.6. Effect of Pharmacological interventions on histopathology

Monocrotaline administered group increased vascular wall thickness due to medial hypertrophy. These features were markedly decreased in treatment groups. Treatment with GSE and punarnava extract normalized medial wall thickness (Fig 10).



(Fig 10)

6. DISCUSSION

In the present study MCT induce pulmonary hypertension has been employed as an experimental model. MCT induced PH is characterized by decrease in endothelium dependent relaxation, oxidative stress, proliferative pulmonary vasculitis, pulmonary hypertension and right ventricular hypertrophy (Mathew et al., 1990; Farkas et al., 2009). Monocrotaline is an 11- membered macrocyclic pyrrolizidine alkaloid. MCT is converted into reactive metabolite in liver and is transported to Red blood cells where it cause endothelial injury and is further specifically reported to cause pulmonary vascular endothelium dysfunction (Madden et al. 1995; Tamosiuniene et al. 2011). Monocrotaline has been reported to reduce endothelium dependent vasorelaxation and nitric oxide bioavailability however exact mechanism has not yet elucidated.

PH consequently causes an increase in cardiopulmonary pressure that leads to increase in RVSP and RVH. Thus increase in RVSP and RVH is used as an indirect parameter of pulmonary hypertension. In the present study cumulative dose response curves were recorded for Ach-induced endothelium dependent relaxation in isolated pulmonary artery ring preparation. Further, Sodium nitroprusside induced endothelium- independent vasorelaxation

has been used as control in the study, to investigate the effect of endothelium independent vascular reactivity. The earlier results suggests that NO is, in fact, the most important endothelium derived mediator involved in Ach-induced relaxation of rat pulmonary artery rings. Endogenous formation of NO is unstable and gets converted to nitrite/nitrate and estimation of serum nitrite/nitrate has been used as an indirect measure of NO release. Thus, the parameter has been used as an index of change in NO formation due to modulation of endothelium function. Increased mRNA expression of eNOS has been shown to stimulate release of NO, therefore reverse transcriptase polymerase chain reaction has been employed to assess the extent of expression of eNOS. Oxidative stress is another important feature of PVED and PH. Thus, TBARS and SO^{2-} were assessed in the present study. Sakaguchi test is a specific test reported in literature for L-Arginine. Thus, this test was performed for the assessment of L-Arginine in the GSE. Further, both GSE and PE have been documented to have antioxidant activities. Thus, free radical scavenging assay (DPPH) was employed in this study to confirm the antioxidant activity of sample obtained.

Bosentan (Ro 47-0203), an orally active non-peptide antagonist of both endothelin receptor subtypes (ETA and ETB has been shown to reduce pulmonary arterial pressure, pulmonary vascular hypertrophy, and right ventricular hypertrophy, without inducing systemic vasodilatation in rats with chronic pulmonary hypertension. It has been reported that ETA subtype of receptors express mainly in the smooth muscle cells, in the vasculature which contribute to vascular constriction and remodeling (Liang et al., 2012).

Combination beneficial effect of herbal extract with pleiotropic effects on cardiac and pulmonary system may attenuate both pulmonary hypertension and resultant cardiac abnormalities. It has been documented that grape seed is a rich source of L-arginine. GSE has also been shown to have vasodilator effects due to presence of L-arginine. This extract have also been shown to activate endothelial nitric oxide synthase (eNOS), up-regulate eNOS in cultured endothelial cells, and cause an endothelium-dependent relaxation (EDR) of blood vessels. It has also been shown that grapes seed extract increase the antioxidant levels and improve the endothelial function (Sivaprakasapillai et al., 2009). It has been reported that punarnava extract, has significant immunomodulatory potential (Rajpoot et al., 2011). L-Arginine has been reported to convert into NO with the help of eNOS. Endothelial NO synthase (eNOS) is a homodimeric flavo-hemeprotein that converts L-arginine to L-citrulline and produces NO intracellularly in response to stimuli. It is believed to be involved in the

regulation of the pulmonary vascular tone and inhibit platelet aggregation. It has an extreme role in the regulation of blood pressure by dwindling the leucocytes adherence and SMC proliferation. NO opposes the actions of potent endothelium- derived contracting factors such as angiotensin II and endothelin-1 (ET-1) (Venema et al., 1999). Nitric oxide inhibits platelet and leucocytes activation and maintains the smooth muscles in a non proliferative state (Cooper et al., 1996). Continuous synthesis of NO by endothelium with in the vasculature also exerts its vasodilator influence on arteriolar tone. Thus GSE and PE combination was employed in the present study for their ameliorative effect in pulmonary hypertension, right ventricular systolic pressure and right ventricular hypertrophy.

MCT administration in rats produces PH assessed in terms of increase RVSP, RV hypertrophy, TBARS level and SO^{2-} levels and decreases Ach induced endothelium dependent relaxation, NO level and eNOS expression. GSE and PE shows significant decrease in RVSP and RV hypertrophy in PVED. GSE obtained as a gift sample was first screened for L-arginine. Both GSE and PE procured as market preparation having antioxidant potential as observed from the DPPH assay. Red colour observed in the test confirms that L-arginine is present in the sample. GSE and PE significantly attenuated RVH and RVSP that shows their beneficial effect on the heart too. Both the extract up-regulates the eNOS expression and ach induced endothelial dependent relaxation that shows the improvement may be done to increase in NO level. Further these extract also reduces the TBARS level and SO^{2-} level exerting the antioxidant potential was also observed into study.Thus, beneficial effect of extract of grape seed and punarnava may be due to increase in eNOS expression and NO synthesis.

CONCLUSION

Thus it may be concluded that Grape seed extract and punarnava extract alone and in combination improves monocrotaline induce pulmonary vascular endothelium dysfunction and pulmonary hypertension by upregulating eNOS expression and nitric oxide production. Thus intervention designed for this pathway may provide therapeutics for pulmonary vascular complication.

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