Research Article

Antihypertensive Efficacy of Methanol Extracts of *Pisonia aculeata* L. on uninephrectomized DOCA - Salt Hypertensive Rats

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ABSTRACT

Pisonia aculeata L. (Nyctaginaceae) is a large thorny climbing shrub, used as a traditional folk medicine. It is used in the treatment of inflammation, pain and oxidative stress associated diseases. The present study has been designed to investigate the anti-hypertensive effect of methanolic extracts of *P.aculeata* on uninephrectomized DOCA-salt induced hypertensive 'Wistar Rats'. Un inephrectomized animals were given 1% NaCl in the drinking water with weekly twice subcutaneous injection of DOCA-Salt (20 mg/kg body wt in olive oil) for six consecutive weeks in order to elevate the blood pressure. Increased TBARS, AST, ALT and ALP and decreased antioxidants including GSH, SOD and GSHPX activity in serum of hypertensive rats were normalized after oral administration of 250 and 500 mg/kg of methanolic leaf extracts of *P.aculeata*. Biochemical assays including triglycerides, cholesterol were also performed to assist the hypothesis. The methanolic leaf extracts of *P.aculeata* also exhibited ACE inhibitory activity. *P.aculeata* significantly decreases blood pressure in a dose dependent fashion as compared to DOCA control rats. The study thus concludes the anti-hypertensive activity of *Pisonia aculeata* in the DOCA –salt hypertensive Wistar rats.

Keywords: Anti-hypertensive activity, *Pisonia aculeata*, uninephrectomy, Doca-salt, Biochemical assays.

INTRODUCTION

One of the key risk factors for cardiovascular disease is hypertension or raised blood pressure. Hypertension or high blood pressure, a chronic medical condition in which the blood pressure in the arteries is elevated. It is summarized by two measurements- Systolic (100-140 mmHg) and Diastolic (60-90mmHg). Hypertension is the most common risk factor for myocardial infarction, stroke, heart failure and peripheral arterial diseases. It is the most common chronic illnesses that the world faces.[1] The relationship between high blood pressure and risk of myocardial infarction is continuous and independent of other risk factors. The higher the blood pressure, the greater is the chance of myocardial infarction and heart failure. [2-3]

There is growing evidence indicating that oxidative stress can precede and contribute to the development of hypertension and its complications.[4-6] Oxidative stress induced by reactive oxygen species (ROS) could be involved in the pathogenesis of hypertension. Oxidative stress, characterized by increased bioavailability of ROS, plays an important role in the development and progression of cardiovascular dysfunction associated with hypertensive disease. Several studies have been reported the increased level of ROS such as superoxide anion, hydrogen peroxide and lipid peroxides in hypertensive patients.[7] Salt-sensitive hypertension is characterized by endothelial dysfunction associated with increase in ROS and local renin-angiotensin-aldosterone system activation.[8] ROS can influence vascular, renal, and cardiac function, contraction/dilatation, and inflammatory responses via redox-dependent signaling pathways. Free radicals may participate in hypertension by damaging target organs through a variety of ways. Of the ROS families, superoxide anion, H_2O_2 and NO are of major importance in the cardiovascular and renal system.[9-10] Therefore, the protection of endothelial from free radical injury is very important in treating hypertension.

Although many new antihypertensive drugs with improved efficacy have been introduced to the market, they still possess serious side effects. Herbal medicines are getting more importance in the treatment of high blood pressure because modern synthetic medicines have side effects. Some of the herbs having antihypertensive potential are *Allium sativum*, *Boerhavia diffusa, Eclipta alba, Gingiber officinalis, Nigella sativa, Rauwolfia serpentina, Vitis vinifera, Withania somnifera* etc. [11]

The plant *Pisonia aculeata* L. holds an important place in folklore medicine. It is extensively used by native medical practioners and tribes for treating swelling, rheumatic pain, Jaundice and tumours. *Pisonia aculeata* is traditionally used in treatment of liver disorder and thought to have a protective effect which may be beneficial to reduce symptoms of hepatotoxicity.[12]*P. aculeata* has been reported to contain several secondary metabolites like flavonoids, steroids, terpenoids, alkaloids, tannins etc.,[13] Previous reports revealed that the leaves of *Pisonia aculeata* exhibited anti-inflammatory, antinociceptive and antioxidant activity and high levels of phenolics and flavonoids in extract may be responsible for its observed biological activities.[14]The literature survey revealed that the anti-hypertensive

activity of *Pisonia aculeata* has not yet been reported so far. Hence the present study was designed to evaluate the anti-hypertensive activity of the plant on DOCA –salt hypertensive rats.

MATERIALS AND METHODS

Collection and preparation of plant extract

Leaves of *Pisonia aculeata* were collected from Kanyakumari District, Tamilnadu. Identification and authentication of the plant species was confirmed at Botanical Survey of India, Coimbatore, Tamilnadu. [Confirmation ID No: BSI/SRC/5/23/2015/Tech/924]. Leaves were thoroughly washed and dried in shade for 10 days. Dried leaves were made into coarse powder using mechanical blender and stored in air tight container till further use. The coarsely powdered leaves of *P.aculeata* were extracted with methanol by following the method as described by Janarthanam and Sumathi (2010).[15]

Experimental Protocol

Animals

Albino Wistar rats weighing 150-200 g were used for the present study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water ad libitum. All the experimental procedures and protocols used in study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC No: KMCRET/Ph.D/15/2016-17)and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drug and Chemicals

DOCA (Deoxycorticosterone acetate) and Dimethyl formamide were purchased from Sigma-Aldrich Chemical Company, Bangalore,India. All other chemicals used in this study were of highest analytical grade obtained from Sisco Research Laboratories (SRL) or Himedia, Mumbai, India.

Experimental Induction of hypertension (Uninephrectomy)

Left Uninephrectomy was performed on all the rats by anaesthetizing with intramuscular injection of Ketamine (20mg/kg.). The kidney was visualized by a left lateral abdominal incision and the left renal artery and ureter were ligated by silk thread, followed by the removal of left kidney. The muscle and skin layer (incision site) were sutured with highly sterile suture needles. After uninephrectomy, rats were allowed to drink tap water ad libitum, with no further treatment. All uninephrectomized animals were given 1% NaCl in the drinking water with weekly twice subcutaneous injection of DOCA-Salt (20 mg/kg body wt in olive oil) for six consecutive weeks (DOCA-salt hypertensive rat). The rats were then, randomly divided into five groups each comprising of six rats.[16]

Experimental work out on DOCA-salt hypertensive rats:

The five groups of the hypertensive rats were divided and categorized into normal control, disease control, positive control and test groups. The animals in the group 1 were not given any surgery or treatment at all, while the group 2 animals were undergone for only surgery and no treatment was done. The animals in group 3 were undergone for surgery and treated with standard drug, Enalapril inj. (48 mg/kg, i.p.) of the total body weight. The animals in the group 4and 5 were undergone for surgery and treated with the plant extracts at 250mg/kg and 500mg/kg of the total body weight. The test animals were treated with the stated dose of plant extract at every 24hr interval, consecutively for 6 weeks. Systolic and diastolic blood pressures were recorded by tail cuff method (IITC, Non-Invasive Blood Pressure Instrument).[17] All the recordings and data analyses were done using computerized data acquisition system and software. At the end of treatment, all the rats were anesthetized with intramuscular injection of Ketamine and sacrificed in CO2 incubator for biochemical assays.

Biochemical assays

At the end of the treatment, after a 12 hr of fast but via access to deionised water, the animals in groups' I-V were sacrificed. Blood samples were collected from each of the animal by retina puncture into plain sterile tubes. Each blood sample was allowed to clot and tubes were subsequently centrifuged at 2000 rpm for 5min to obtain sera which was transferred into new tubes and kept at -20^{0}C until used for bioassays using Erba 360 – fully automated clinical chemistry analyser.[17-18]

Determination of biochemical parameters

For assessment of liver function, blood samples were collected from the animals by puncturing the retro-orbital plexus and centrifuged. The serum collected after centrifugation was analyzed for various biochemical parameters like SGOT, SGPT and ALP. Serum transaminase activity was measured according to the method of Rietman and Frankle[19] and ALP was determined by using method of Kind and king.[20]

Antioxidant enzymes viz. Superoxide dismutase (SOD),[21] Catalase (CAT),[22] Glutathione peroxidise (GPX),[23] Reduced glutathione (GSH)[24] and Lipid peroxidation (LPO)[25] were determined in all the liver tissues of all the tested rats . Thiobarbituric acid reactive substances (TBARS) levels in the tissues were determined by the method described by Buege & Aust with slight modifications.[26]The total cholesterol, Triglyceride, HDL and LDL contents were determined enzymatically.[27-28] Serum peroxynitrate level was determined according to the method described by Moorcroft *et al*., [29]

Angiotensin converting enzyme (ACE) inhibition assay:
Serum ACE activity was measured using Hippuryl-His-Leu (HHL) as a synthetic substrate. After 7, 14 and 21 days of treatment, blood was collected and serum was separated. Then 100 µl of rat serum was added to 150 µl of HHL (5 mM) in phosphate buffered saline (NaCl 300 mM) at pH 8.3. Test and control tubes were incubated for 30 min at 37°C with shaking. The enzymatic reactions were terminated by the addition of 0.25 ml of 1N HCl; HCl was added before the serum in 0-time control assays. The hippuric acid formed by action of the ACE on HHL is extracted from acidified solution into 1.5 ml of ethyl acetate by vortex mixing. After a brief centrifugation, 1 ml aliquots of each ethyl acetate layer was transferred to a clean tube and heated at 120°C for 30 min. The hippuric acid was then re-dissolved in 1 ml distilled water and the amount formed was determined from its absorbance at 228 nm using ultraviolet (UV) spectrophotometer.[30]

Statistical analysis
All the data were expressed as means ± SEM (n=6). One way ANOVA, followed by Dunnett's tests were performed. P values< 0.05 were considered significant.

RESULTS AND DISCUSSIONS

The long-term administration of DOCA-salt induces sodium retention and in the presence of a high salt, it produces volume-dependent type of hypertension in rats.[31] In the present study, the systolic and diastolic blood pressure significantly increased in DOCA-salt rats. Oral administration of the *Pisonia aculeata* leaf extracts (250 and 500mg/kg) and standard Enalapril resulted in a significant reduction in systolic and diastolic blood pressure. The Blood pressure is controlled by a number of different biochemical pathways.

Effect of methanolic leaf extracts of *Pisonia aculeata* on serum SGOT, SGPT and ALP activity in experimental rats

The activities of serum hepatic marker enzymes (AST, ALT and ALP) in the control and experimental animals were assayed. The activities of hepatic marker enzymes increased in DOCA salt-induced rats and treatment with extracts (250 and 500mg/kg) and standard Enalapril significantly restored the activities of the marker enzymes (Figure 1).

AST, ALT and ALP are the relatively liver specific enzymes. Their estimation in the serum is useful as a quantitative marker of the extent and type of liver damage. In our results, AST, ALT and ALP activities were increased considerably in the serum of DOCA-salt hypertensive rats (group 2), which is a clear evidence for liver damage. The reason behind this elevation may be due to the necrotic and oxidative action of liver tissues which causes leakage of these enzymes from hepatocytes as a result of membrane damage.[32]

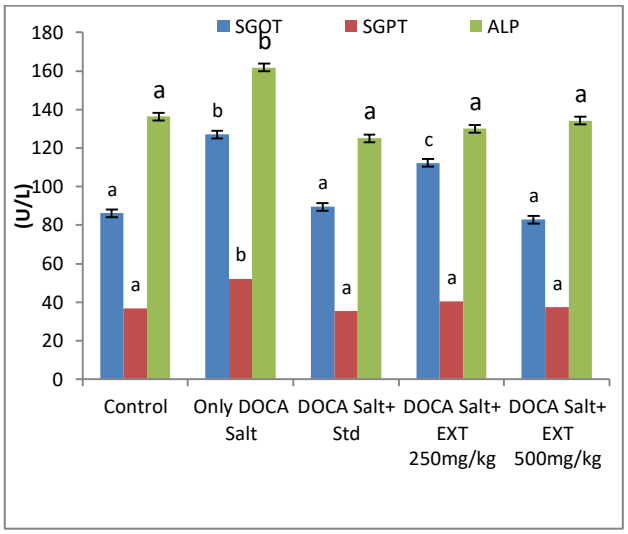


Figure 1: Effect of methanolic leaf extracts of *P. aculeata* on serum SGOT, SGPT and ALP activity in experimental rats

The reduced antioxidant status might also be involved in the hepatic injury because reactive free radicals are implicated as the potential mediators of tissue injury. In this study, the activities of these enzymes were found to increase in the group where hypertension was induced using DOCA-salt, but were significantly reduced in groups that received extracts and standard. The results obtained by Alamgeer *etal* in Pharmacological evaluation of antihypertensive effect of aerial parts of *Thymus linearis* Benth also states that the extract (500 mg/kg) produced a significant reduction in serum ALT, AST and ALP levels and the reduction of these enzymes indicated that the extract did not cause any toxic effects on both liver and heart tissues .[33]

Effect of methanolic leaf extracts of *Pisonia aculeata* on liver Superoxide dismutase, Catalase, Glutathione Peroxidase, GSH, Lipid peroxidation and TBARS levels in experimental rats

The activities of SOD, CAT, GPx and reduced Glutathione decreased significantly(Figure 2 and 3) , where as lipid peroxidation and TBARS levels increased significantly in DOCA-salt rats and the administration of extracts and standard Enalapril significantly restored these parameters as shown in Figure3 and 4.

SOD, CAT and GPx are major free radical scavenging enzymes that have shown to be reduced in a number of pathophysiological processes and diseases such as hypertension.[34] In the present study, DOCA-salt rats caused a significant depletion of enzymatic antioxidants in erythrocyte and tissues. SOD (Superoxide Dismutase) is an enzymatic antioxidant which reduces superoxide radical to hydrogen peroxide. CAT (Catalase) is a heme protein located predominantly in peroxisomes and the inner mitochondrial membrane that catalyzes the conversion of H_2O_2 to water and molecular oxygen.[35]

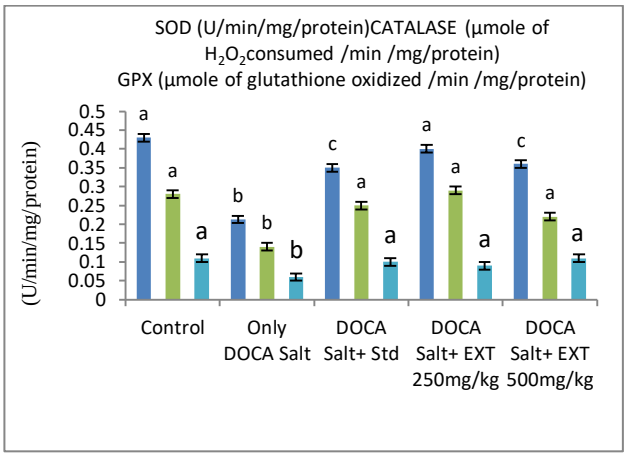


Figure 2: Effect of methanolic leaf extracts of *P. aculeata* on liver SOD, Catalase, Glutathione peroxidise levels in experimental rats.

A decrease in the activities of these antioxidant enzymes in tissue leads to the formation of superoxide anion and hydrogen peroxide which can later form hydroxyl radical. GSH (Reduced Glutathione)-metabolizing enzyme, GPx(Glutathione peroxidise) work in concert with glutathione in the decomposition of hydrogen peroxide and other organic hydro peroxides to non-toxic products. Reduced activity of GPx was observed due to inactivation of this enzyme by ROS. Treatment with extracts (250 and 500mg/kg) show increased activities of these enzymatic antioxidants, which might be due to the presence of phenolic compounds. Thus, administration of this extract clearly shows the free radical scavenging activity, which could exert a beneficial action against pathophysiological alterations caused by superoxide anion and hydroxyl radicals. The relationship between the development of hypertension and the increased bioavailability of ROS or decreased antioxidant capacity or both have been demonstrated in many experimental models of hypertension.[36]

Non-enzymic antioxidants such as reduced glutathione play an excellent role in protecting the cells from oxidative damage. GSH is one of the most important endogenous antioxidants. It plays the role of a sulfhydryl (SH) group provider for direct scavenging reactions. Glutathione peroxidase (GPx) catalyses peroxide reduction utilizing GSH as the substrate. Decreased GSH concentration may also contribute to decreased GPx activity because GSH is one of the substrates for GPx.[37]In our study, the plasma and tissue GSH concentration significantly decreased in DOCA-salt rats which may be due to an increased utilization of GSH. The treatment with extracts (250 and 500mg/kg) has elevated the levels of these parameters in DOCA-salt rats may be responsible for the decreased level of lipid peroxidation.

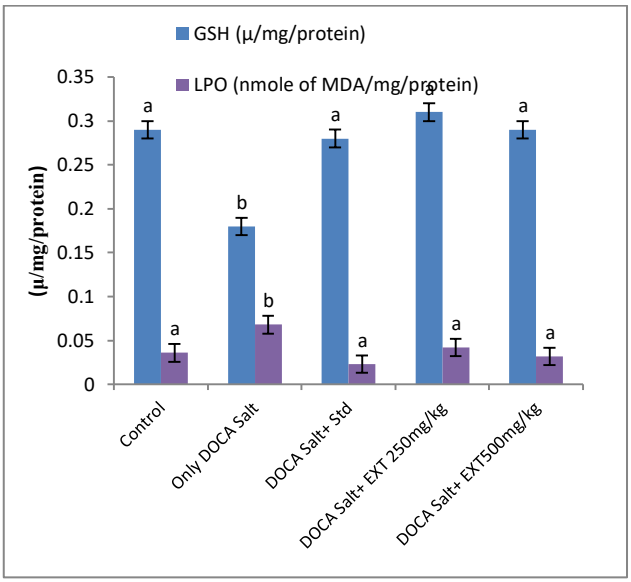


Figure 3: Effect of methanolic leaf extracts of *P. aculeata* on Reduced Glutathione and Lipid Peroxidation Level in experimental rats.

In this study, the concentration of TBARS (Thiobarbituric Acid-Reactive Substances) and LPO (Lipid Peroxidation Level) significantly increased in the plasma and tissues of DOCA-salt rats as reported earlier in clinical and experimental hypertensive rats.[38]The role of TBARS in DOCA induced hypertension could be due to direct anti-oxidant and free radical scavenging activity of *P.aculeata*. Studies have shown that consumption of anti-oxidants like *Emblica officinalis* protect against oxidative stress-augmented pathophysiological abnormalities including hypertension.[39]The increased concentration of lipid peroxidative markers suggests an increase in oxygen free radicals.[40] The levels of lipid peroxidative markers in extracts (250 and 500mg/kg) treated rats decreased significantly, which might be due to the presence of phenolic compounds such as coumarins, flavonoids, steroids and triterpenes .[41-42]

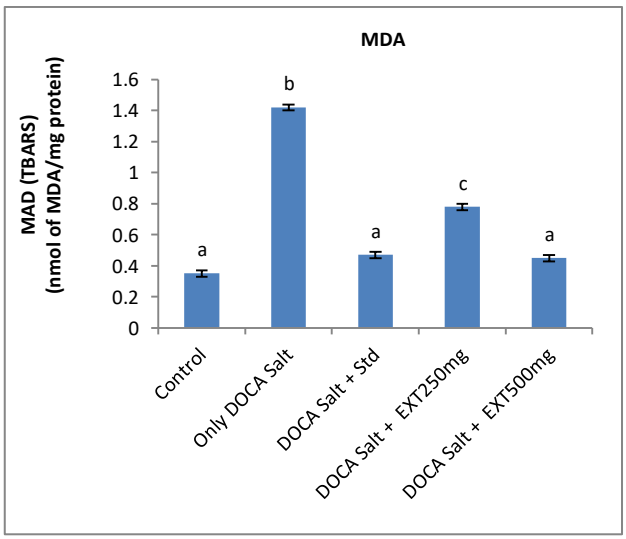


Figure 4: Effect of methanolic leaf extracts of *P. aculeata* on Malondialdehyde (MDA) content in experimental rats.

Effect of methanolic leaf extracts of *Pisonia aculeata* in serum Total Cholesterol, Triglycerides and HDL - Cholesterol, LDL- Cholesterol level in experimental rats

The effect of leaf extracts (250 and 500mg/kg) and standard Enalapril on Total cholesterol, triglycerides, HDL-C and LDL-C in the plasma of DOCA salt hypertensive rats were examined. Extracts and standard Enalapril administration exhibited significant reduction in Total cholesterol, triglycerides, LDL-C and elevation in HDL-C. (Figure 5).

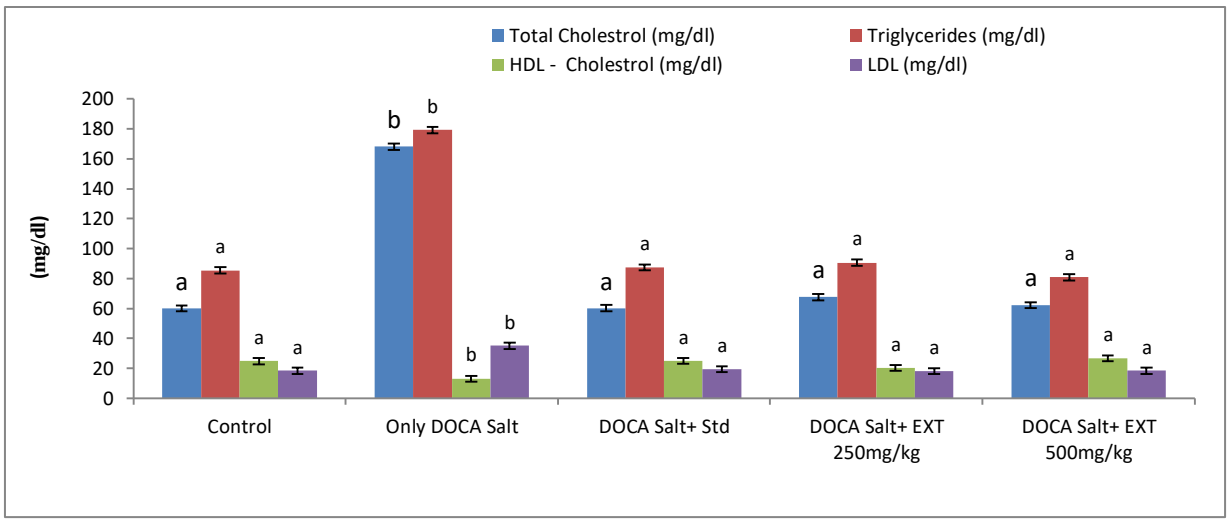


Figure 5: Effect of methanolic leaf extracts of *P. aculeata* in serum Total Cholesterol, Triglycerides and HDL - Cholesterol, LDL- Cholesterol level in experimental rats

In this study, we observed a higher concentration of Total cholesterol in DOCA-salt hypertensive rats. The Extract exhibited significant decrease in the levels of TC, triglycerides, LDL levels and increase in HDL levels as compared to the control. This indicates a possible reduction in cardiovascular risk factor and could be responsible for its anti-hypertensive effect. The present result coincides with the studies done in *Thymus linearis* where the anti-hypertensive effect is associated with its lipid lowering effect.[33]

Effect of methanolic leaf extracts of *Pisonia aculeata* on Peroxynitrate in experimental rats

The effect of extracts (250 and 500mg/kg) on Peroxynitrate level of DOCA-treated rats were investigated. Peroxynitrate level of DOCA-salt-treated hypertensive rats was significantly higher than the control. Supplementation of extracts and standard Enalapril to the hypertensive rats produced significant decrease in the peroxynitrate level as shown in figure 6.

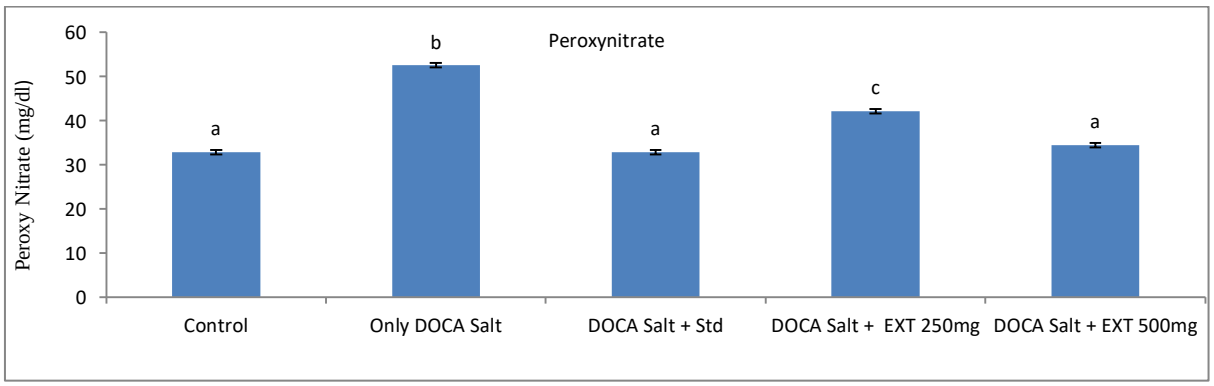


Figure 6: Effect of methanolic leaf extracts of *P. aculeata* on Peroxynitrate in experimental rats.

The endothelial cell which is recognized as a source of NO has also been identified as a potential site of ROS production.[43-44] The initial hypothesis for deleterious effects of NO has been based on its free radical nature and its reactivity. NO diffuses out and can reach adjacent cells, where it reacts with several important enzymes from the mitochondrial electron transport chain. Reactive oxygen radicals may inactivate NO by converting them in to peroxynitrite with superoxide anion thereby causing arteriolar vasoconstriction.[45] Superoxide radicals in and around vascular epithelial cells play a critical role in the pathogenesis of hypertension. ROS may decrease NO bioavailability and impair diastolic function.[46]Studies with No- donor drugs suggest that overproduction of NO in the human heart might impair diastolic relaxation. It remains to be determined, why normal production of NO is protective in cardiovascular system whereas over production of NO is potentially harmful.[47]

Effect of methanolic leaf extracts of *Pisonia aculeata* on lung ACE (Angiotensin-converting enzyme) levels in experimental rats

The effect of leaf extracts (250 and 500mg/kg) on Angiotensin-converting enzyme (ACE) activity of DOCA-treated rats were investigated. The Angiotensin-converting enzyme (ACE) activity of DOCA-salt-treated hypertensive rats was significantly higher than the control. Supplementation of extracts and standard Enalapril to the hypertensive rats produced significant decrease in the activity of ACE as shown in figure 7.

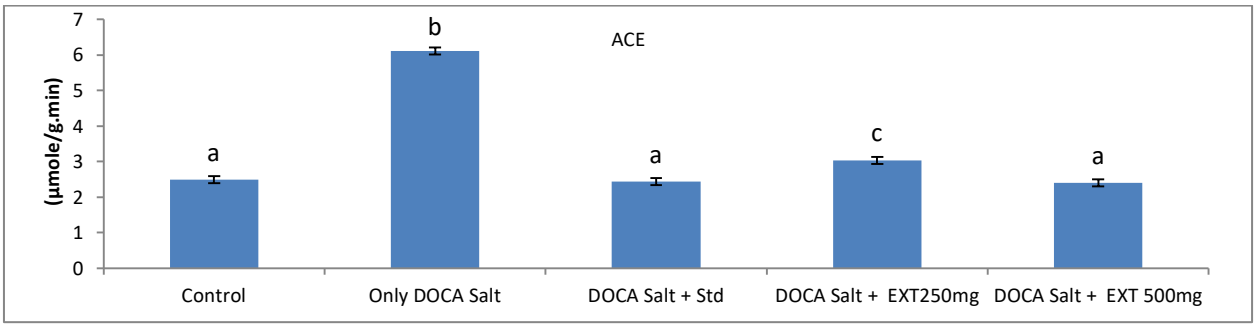


Figure 7: Effect of methanolic extract of *P.aculeata* on lung ACE Levels in experimental rats.

Angiotensin converting enzyme (ACE), a key component of the Renin-Angiotensin Aldosterone System (RAAS) plays an important role in blood pressure regulation. ACE plays a significant role in converting Angiotensin I (Ang I) to Angiotensin II (Ang II), a potent vasoconstrictor implied in the development of important cardiovascular risk factors like hypertension. The pathogenesis of hypertension could be due to many reasons like RAAS, sympathetic nervous system, genetic influence etc., among them over activation of RAAS is significant.[48]Therefore inhibition of ACE is a promising way controlling over expression of RAAS.

Different types of natural food derived compounds have been investigated on their ACE inhibitory properties. Some terpenoids and polyphenolic compounds including flavonoids, xanthones etc are found to be effective as natural ACE inhibitor.[49-50]

When a bioassay –guided fractionation of extract of *Sedum sarmentosum* was performed, five purified flavanols were found to possess ACE inhibitory activity.[51] Flavonoids are one of the major groups of plant secondary metabolites with numerous beneficial pharmacological properties. The preliminary phytochemical studies on methanolic extracts of *Pisonia aculeata* showed the presence of Quinones, Terpenoids, Steroids, Tannins, Coumarins, flavonoids, Phenols etc.,[52] Most studies have showed that plant extracts rich in phytochemicals found to be effective in ACE inhibition. The present study also proved to be effective in suppressing the activity of ACE.

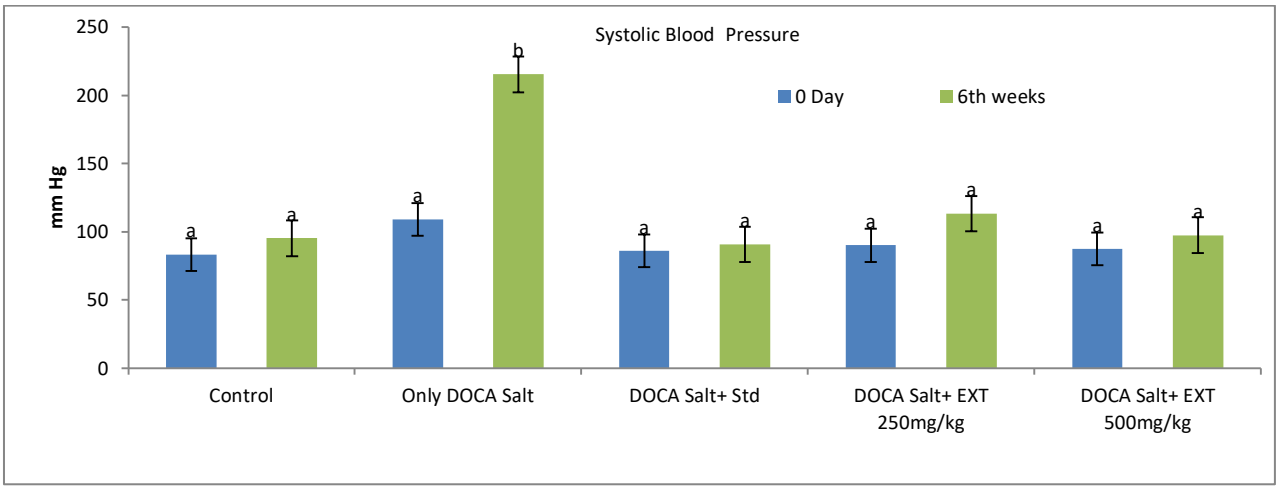
Effect of methanolic extract of *P.aculeata* on Systolic and Diastolic Blood Pressure in experimental rats

The effect of leaf extracts on systolic and diastolic blood pressure of DOCA-treated rats was investigated. The systolic and diastolic blood pressure of DOCA-salt-treated hypertensive rats was significantly higher than the control, administration of extracts (250 and 500mg/kg) and standard Enalapril to the hypertensive rats produced significant lowering effects on the blood pressure. (Figure 8)

An increased concentration of aldosterone leads to increased re-absorption of sodium ions and water from kidney, thereby influencing the blood pressure levels.[53] The increased aldosterone may activate oxidative stress in the Doca –salt model.[54]In agreement with the previous reports,[55] we also noticed that systolic and diastolic blood pressure were considerably increased in Doca-salt hypertensive rats. This might be due to increased oxidative stress. Oral adminstration of plant extract resulted in significant reduction in blood pressure which is due to the anti-hypertensive property of the plant.

CONCLUSION

The present study demonstrates significant anti-hypertensive effect of methanolic extracts of *Pisonia aculeata.* Previous studies states that there is a proposed relation between the pathogenesis of hypertension and cardiovascular diseases to oxidative stress. In line with various other important studies our data support an imperative role for oxidative stress in the pathogenesis of hypertension as well as its complication , cardiac and renal hypertrophy in DOCA treated rats. In our study , *P.aculeata* mediated attenuation of oxidative stress in DOCA induced hypertension could be due to direct antioxidant and free radical scavenging activity of *P.aculeata*.



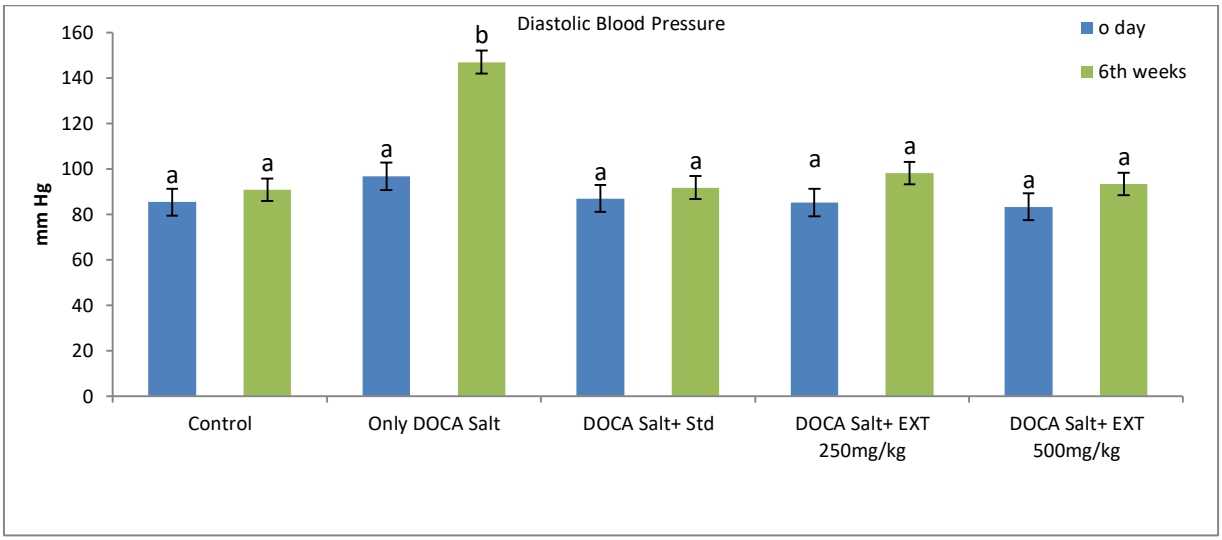


Figure 8: Effect of methanolic extract of *P.aculeata*on Systolic and Diastolic Blood Pressure in experimental rats.

P. aculeata has a long history of use in various ailments in the traditional system of medicine. It has been reported to be safe as it did not cause any lethality or adverse changes up to the dose of 2000 mg/kg b.wt. Its extract contains different phytochemicals. The observed effect may be the combined effect of these constituents.

In conclusion our data demonstrates the chronic administration of methanolic leaf extracts of *Pisonia aculeata* reduces elevated blood pressure with maximum effect at 500 mg/kg body weight of extract. However clinical trials are needed to document the role of *Pisonia aculeata* in the treatment of hypertension.

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