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Original Research Article

Aqueous Extract of *Emilia praetermissa* Milne-Redh. (Asteraceae) Leaf Attenuates Salt-induced Hypertension in Male Wistar Rats: Biochemical Evidence

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ABSTRACT

Emilia praetermissa is a herb consumed for folkloric management of hypertension. However, its efficacy against hypertension has not been scientifically investigated. This study evaluated the antihypertensive effects of its aqueous leaf extract in salt-induced hypertensive male Wistar rats. Seven groups (n=6 each) were treated: Group 1 (control), Group 2 (8% NaCl for hypertension induction), Group 3 (extract alone at 100 mg/kg), Group 4 (extract before salt-loading), Group 5 (salt-loading before extract), Group 6 (salt-loading followed by captopril at 50 mg/kg), and Group 7 (salt-loading followed by hydrochlorothiazide (HCTZ) at 10 mg/kg). All treatments were administered orally by gavage daily for two weeks. Blood pressure measurements—systolic (SBP), diastolic (DBP), mean arterial pressure (MAP), and heart rate (HR)—were recorded using the tail-cuff method. The extract's oral acute toxicity (LD_{50}) exceeded 5000 mg/kg. At 100 mg/kg, it significantly ($p \leq 0.05$) reduced blood pressure in hypertensive rats, improved liver and kidney function indices ($p \leq 0.05$), lipid profiles ($p \leq 0.05$), and antioxidant enzymes levels in heart tissue ($p \leq 0.05$). Angiotensin II (AngII) and aldosterone (ALD) levels decreased significantly ($p \leq 0.05$), while 6-keto-prostaglandin $F_{1\alpha}$ increased ($p \leq 0.05$). Histopathological analysis revealed that the extract improved myocardial fibrosis and reduced tunica media thickness in the heart and aorta, while also mitigating ultrastructural changes in the kidney and liver of hypertensive rats. The extract demonstrated a significant antihypertensive effect in 8% NaCl-induced hypertensive male rats. These results provide scientific support for the traditional use of *Emilia praetermissa* in managing hypertension.

Keywords: *Emilia praetermissa*, Hypertension, Angiotensin II, Aldosterone, Oxidative stress.

Introduction

Hypertension is one of the non-communicable diseases that have become a burden in developing countries.[1,2] The continent of Africa bears a disproportionate burden of this increase due to poor preventive and expensive health schemes.[3,4] It has been predicted that 75% of the world's hypertensive population will emerge from developing countries by 2025.[5] Nigeria bears a substantial burden of the total number of persons suffering from hypertension in Africa because of the large population of the country which is currently estimated to be over 200 million.[6] Untreated hypertension results in complications such as cardiovascular diseases, kidney failure, stroke, blindness, and nerve damage. [2,7]The renin-angiotensin system (RAS) plays an important role in the development and progression of hypertension and hypertension-related damage to cardiovascular organs.[8] Angiotensin II constricts vascular smooth muscle, promotes aldosterone production, stimulates catecholamine release, prevents sodium and water loss in the kidneys, and exacerbates the remodelling of cardiovascular organs.[9,10]

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It has been shown that inhibition of the production of angiotensin II by ACE inhibitors can produce antihypertensive effects as well as protection of the cardiovascular organs from damage.[11,12] Recently, efforts have been made to find new drugs that possess ACE inhibitory activity.[13] ACE activity and that of its product angiotensin II are established hypertension-inducing agents.[14] Inhibitors of RAS are the main antihypertensive drugs in clinical practice, but come with some potential side effects such as hypotension, hyperkalaemia, cough, angioedema, and renal impairment, as well as some limitations such as variability in response, tolerance and resistance, teratogenicity, and elevated serum creatinine levels. Over the years, the use of plant parts (herbs) for the treatment of numerous human diseases [15], has necessitated increased scientific scrutiny of the therapeutic potential and safety of these plant parts.[16] One of such plant is *Emilia praetermissa* (*Ep*) which belongs to the family of Asteraceae. *Emilia praetermissa* was originally described in the literature as a plant first encountered in Sierra Leone and Nigeria [17] and was later found in other West African countries, including Cameroon, Côte d'Ivoire, Ghana, Guinea, and Liberia. [18, 19, 20] Its common name is Kipo or Koyagipo.[21] *Emilia praetermissa* is an erect annual plant that usually grows up to 1-meter-tall, but occasionally to 1.5.[22] The common name of *Emilia praetermissa* Milne-Redh. (Asteraceae) is yellow thistle while its local names in Yoruba and Igbo is 'Odundun-odo' and 'Nti-ene see' respectively.

Its habitat is open ground in urban areas near buildings and the forest zone. It is also commonly found in brackish mangrove swamps.[22] The plant (leaves) is sometimes harvested from the wild by local folks for food and medicinal purposes. The green leaves are crushed and used externally to treat sores and sinusitis and as a poultice for wounds. The

leaves are also mixed with those of *Ipomoea eriocarpa*, and soaked in cold water and the resulting infusion is used as eye drops. [23]

The roots are also used to treat colic in babies and as a medication for chest ailments. Most importantly, the leaves are known for their lipid-lowering effect [24] and are macerated to and used as cardioprotective. [21] *Emilia praetermissa* leaf is believed to contain bioactive agents capable of lowering blood pressure. Folks in Nigeria, pluck the fresh leaves and chew them for that purpose. The present study was therefore designed and undertaken to evaluate *Emilia praetermissa* leaf for antihypertensive potential using salt-induced hypertensive rats as a model and to give a scientific basis to *Emilia praetermissa* in the future management of hypertension.

Materials and Methods

Animals
A hundred (100) adult male Wistar albino rats 150 ± 10 g and about 12 weeks old were purchased from the Department of Biochemistry, University of Benin Edo-State Nigeria. They were housed in wood-framed iron mesh cages in the animal house of the Department of Pharmacology and Toxicology, University of Benin in an environmentally controlled room with a 12-h light: 12-h dark cycle and left to acclimatize for seven (7) days. They were maintained on growers' mash and water *ad libitum* throughout the experimental period. All experiments were performed in compliance with the guidelines for the care and use of laboratory animals approved by the Animal Ethics Committee of the University of Benin Edo-State Nigeria (approval number LS19106)

Preparation of plant extract
Fresh leaves of *Emilia praetermissa* were collected from the University of Benin, Ugbowo Campus, Benin-City, Edo State in the months of July-September 2018. It was checked on http://www.worldfloraonline.org on the 11th of June, 2018. The plant material was identified by Dr. H.A. Akinnibosun and substantiated in the Department of Plant Biology and Biotechnology (PBB), Faculty of Life Sciences, University of Benin, and a herbarium number (the University of Benin Hebarium *EMILIA* 407) UBHE 407 was obtained. The leaves were rinsed with distilled water to remove dust and other foreign particles and air-dried completely. The dried leaves were milled into a powder using a clean mechanical blender and stored in a sterile air-tight container until required. The powdered material (100 g) was infused for 72 hours in distilled water (500 mL) with intermittent agitation. The suspension was sieved through a strainer and subsequently filtered using No 1 Whatman filter paper. The filtrate was stored in a beaker, put on the 'material tray' of a freeze dryer and pre-frozen. Immediately after the pre-freezing process was over, the drying process was begun by transferring the frozen filtrate onto the drying shelf of the freeze dryer and allowed to dry at ≤ -40ºC for 24 hours. The lyophilized (freeze-dried) extract was stored at 4°C in an air-tight glass container until required for subsequent aspects of this study.

Qualitative phytochemical analysis of aqueous extract of Emilia praetermissa
Phytochemical screening of the aqueous extract was carried out to identify secondary metabolites. The phytochemicals assayed for were alkaloids [25] flavonoids [26] saponins [25] and tannins. [27]

Acute toxicity study
In this study, the method described by [28] was adopted to determine LD_{50}. A total of twelve (12) male Wistar rats weighing (150 ± 10 g) were used. The rats were allowed one week of acclimatization period. This study was conducted in phases. In phase I, a total of nine (9) male Wistar rats were used. The Wistar rats were divided into three (3) groups of three (3) rats each with each group receiving 10, 100, and 1000 mg/kg body weight respectively of aqueous leaf extract of *Emilia praetermissa* via gavage and were observed for 24 hours for signs of behavioural changes and (or) mortality. In the post-administration phase II, a total of three (3) male Wistar rats were used and divided into three (3) groups of one (1) rat each, each group administered doses of 1600, 2900, and 5000 mg/kg body weight respectively of the extract respectively. The animals were observed for another 24 hours for signs of behavioural changes and (or) death associated with toxicity.

The lethal dose (LD_{50}) of aqueous leaf extract of *Emilia praetermissa* was calculated as shown below:

$$LD_{50} = \sqrt{D_0 * D_{100}}$$

D_0 = Highest dose that resulted in no death; D_{100} = lowest dose that resulted to death.

Preparation of 8% sodium chloride solution for induction of hypertension
Sodium chloride (8 g) was dissolved in 100 mL of distilled water. Hypertension was induced by salt-loading rats with 8% sodium chloride solution as drinking water for 1 week and 0.9% sodium chloride solution for another 2-3 weeks according to the procedure elucidated by Baydal et al. [29] with slight modifications. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) were measured by the tail-cuff method and recorded. Rats with systolic blood pressure and diastolic blood pressure above 140- and 100-mm Hg respectively after three consistent readings were considered hypertensive.

Effect of aqueous extract of Emilia Praetermissa leaf on blood pressure parameters in hypertensive rats: Dose response study
The antihypertensive effects of varying doses of the aqueous extract of *Emilia praetermissa* leaf were evaluated in salt-induced hypertensive male rats following a single oral administration. Eighteen (18) male rats were randomly assigned into three groups of 6 rats each. The hypertensive rats in groups 1, 2, and 3 received single oral doses of 50, 100, and 200 mg/kg body weight, respectively. Systolic, diastolic, and mean arterial blood pressures, along with heart rates at baseline (0 hrs.), and at 1- and 3-hours post-administration, were measured using the non-invasive tail-cuff method.[57] The dose that demonstrated a significant antihypertensive effect, characterized by pronounced reductions in systolic, diastolic, and mean arterial blood pressures within 3 hours, was identified for further study.

Animal treatment
Rats in Group I (Control) were given normal commercial rat pellets and water *ad libitum*. Group II (8% NaCl) rats were maintained on commercial rat pellets and salt-loaded (8% sodium chloride solution as drinking water). Group III *Ep* (100 mg/kg) rats were maintained on commercial rat pellets and given 100 mg/kg body weight of aqueous extract of *Emilia praetermissa* leaf, Group IV (*Ep* 100 mg/kg + 8% NaCl) rats were maintained on commercial rat pellets and given 100 mg/kg body weight of aqueous extract of *Emilia praetermissa* leaf for two (2) weeks prior to salt-loading (8% NaCl solution as drinking water). Group V (8% NaCl + *Ep* 100 mg/kg) rats were maintained on commercial rat pellets and salt-loaded (8% NaCl solution as drinking water) prior to treatment with 100 mg/kg body weight of the aqueous extract of *Emilia praetermissa* leaf for two (2) weeks. Group VI (8% NaCl + Captopril 50 mg/kg) and VII (8% NaCl + HCTZ 10 mg/kg) rats were maintained on commercial rat pellets and salt-loaded (8% NaCl solution as drinking water) prior to treatment with standard hypertensive drugs captopril (50 mg/kg body weight) and hydrochlorothiazide (10 mg/kg body weight) respectively for a period of two (2) weeks.

In the fifth week, the rats were anesthetized intraperitoneally with 25% urethane (1.0 g/kg body weight) in saline. The abdominal and thoracic cavities were opened and blood samples were collected by cardiac puncture and put in heparinized sample bottles. The liver, kidney, heart, and aorta were excised and stored at 4ºC for biochemical analysis, while specimens for histological examination were placed in formalin at room temperature.

Biochemical analysis
Plasma samples were used to measure liver function marker enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) according to the method of Reitman and Frankel[30] and gamma

glutamyl-transferase (GGT) according to Szasz[31] and kidney function index molecules: urea and creatinine according to the method of Fawcet and Scott[32] and Bartels and Bohmer[33] respectively. Chloride ion, sodium ion, and potassium ion according to.[34] Bicarbonate ion according to the method of Tietz.[35] Lipid profiles including total cholesterol, triacylglycerol, and high-density lipoprotein were estimated based on the methods of Allian et al,[36] Fossati and Prencipe,[37] Grove ,[38] and Burstein et al.[39] Low-density lipoprotein and very low-density lipoprotein levels were calculated by using the method of Friedewald. [40] Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities as well as reduced glutathione (GSH) and malondialdehyde (MDA) levels assays were based on the methods of Misra and Fridovich, [41] Cohen et al.[42] Tietze, [43] Flohe and Guuzler, [44] and Ohkawa et al. [45] respectively. Angiotensin II (Ang II), aldosterone (ALD), and 6-keto-Prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) levels were measured using commercial ELISA kits.

Determination of blood pressure parameters
A commercially available automated computerized tail-cuff blood pressure monitor, the IITC Life Science MRBP recording system was used to record the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) in rats. Rats were placed in a pre-heated restrainer with a head gate, several minutes (5-15 minutes) prior to the commencement of the test in order for each rat to acclimatize sufficiently.[57] The restrainer was then placed in a thermostatically controlled warming chamber (32±2ºC) to warm the rat's tail and maintain blood flow, for effective detection of pulse in the tail artery. The blood pressure of the rats was thereafter monitored and an average of three (3) consistent readings were taken for each rat.

Preparation of tissue homogenate
A piece (1g) of heart from each rat was homogenized in 9 mL of cold phosphate buffer (0.05 M and pH 7.0) using a homogenizer fitted with a Teflon pestle, mechanically. The homogenate was centrifuged for 10 minutes at 4000 rpm. The separated supernatant was kept frozen at -20°C until they were needed for biochemical assays.

Histopathological examination
The liver, kidney, heart, and aorta were fixed in neutral buffered formalin. The fixed organs were completely dehydrated using absolute ethanol followed by 96% ethanol, and 70% ethanol, and then rinsed with distilled water. A 5 µm section was prepared in each case and stained using Periodic Acid-Schiff (PAS) stain, Masson Trichrome (MT) stain, and Verhoeff-Van Giesson (VVG) stain for ultra-structural changes occasioned by salt-loading induced hypertension and treatment with the extract. The stained tissues were viewed using an optical photomicroscope (Leica DM 500, Leica Biosystems, Germany) at x 400 magnification.[46]

Statistical analysis
Data are presented as mean ± standard error of the mean (SEM). Significant differences were determined using a one-way analysis of variance (ANOVA). Differences between means were analyzed for significance using Dunnett's multiple-range tests. ANOVA and Dunnett's multiple range tests were based on the computer software, graph pad prism, version 7. Values of $p \leq 0.05$ were considered statistically significant.

Results and Discussion

Phytochemical constituents of aqueous extract Emilia Praetermissa leaf
Phytochemical analysis of aqueous extract of *Emilia praetermissa* leaf qualitatively, confirmed the presence of alkaloids, flavonoids, saponins and tannins. This finding is similar to the study carried out by Ngozi et al.[47] The phytochemical analysis of the extract confirmed the presence of alkaloids, flavonoids, saponins, and tannins, which are known for their therapeutic properties. Alkaloids exhibit anti-inflammatory, analgesic, and antihypertensive effects [48, 49] while flavonoids provide antioxidant, anti-inflammatory, and cardioprotective benefits. [50, 51] Saponins lower cholesterol, support the immune system, and help manage hypertension. [52, 53] Tannins contribute to wound healing, inflammation reduction, and antimicrobial protection. [54] Overall, these compounds suggest that *Emilia praetermissa* may have potential therapeutic applications, particularly in treating oxidative stress, inflammation, and hypertension.

Acute toxicity test
The rats treated with varying doses of the extract (10, 100, 1000, 1600, 2900, and 5000 mg/kg body weight) did not show any physical signs of toxicity caused by the extract during the entire experiment and no mortality was recorded after 72 hours. The findings of this study suggest that *Emilia praetermissa* leaf extract is relatively safe. The absence of observable toxic effects or behavioural changes across the administered doses indicates that the extract's LD_{50} may exceed 5000 mg/kg, placing it in the "non-toxic" category according to OECD guidelines.

Antihypertensive effects of aqueous extract of Emilia Praetermissa leaf on hypertensive rats after single administration at varying doses
After single administration of 50, 100, and 200 mg extract /kg body weight the systolic, diastolic, and mean arterial pressure were significantly reduced ($p \leq 0.05$) after one hour and continued to decrease for 3 hours, compared to the initial systolic, diastolic and mean arterial pressure of the salt-induced hypertensive rats (Tables 1). There was a significant decrease ($p \leq 0.05$) in heart rates of the hypertensive rats administered a single dose of 50 mg extract/kg body weight. The extract also tended to decrease heart rates of the hypertensive rats given single doses of 100 and 200 mg extract/kg body weight after one hour compared to initial heart rates ($p > 0.05$). There were increases in heart rates of rats exposed to single dosages of 50 and 100 mg extract/kg body weight sequentially at the end of the 3 hours but were not statistically significant ($p > 0.05$). The observation in rats administered 200 mg extract/kg body weight was different. This group of hypertensive rats exhibited a continued decrease in heart rate (Table 1). The extract exhibited a significant antihypertensive effect after a single dose in hypertensive rats, resulting in marked reductions in systolic, diastolic, and mean arterial blood pressures within 3 hours. The effective dose was identified as 100 mg/kg body weight. These findings indicate that the extract may exert blood pressure lowering effect, particularly at a dose of 100 mg/kg, in hypertensive rats without causing notable alterations in heart rate. This finding is consistent with the effects observed following a single administration of hydro-ethanol extract of *Plantago asiatica* seeds in spontaneously hypertensive rats. [55]

Effect of Emilia praetermissa on liver function status in normotensive and hypertensive rats
Plasma ALT, AST, ALP, and ɤ-GT activities were measured to investigate the effects of the aqueous extract of *Emilia praetermissa* leaf on liver function. As shown in Table 2, the activities of liver marker enzymes (ALT, AST, and ɤ-GT) were significantly elevated ($p \leq 0.05$) in the plasma of the salt-loaded rats compared to control, extract treated normotensive, pre-treated salt-loaded, and the salt-loaded rats treated with extract and standard drugs respectively. ALP was significantly decreased ($p \leq 0.05$) in the plasma of the salt-loaded rats' group compared to control, extract-treated normotensive, and salt-loaded rats treated with standard drugs. However, compared to pre-treated salt-loaded and salt-loaded rats treated with extract; the decrease was not statistically significant ($p > 0.05$). For the liver secretory function parameters, there was a slight increase in total bilirubin level in the salt-loaded rats compared to control, extract-treated normotensive, pre-treated salt-loaded and salt-loaded rats treated with extract and standard drugs. However, this increase was not statistically ($p > 0.05$) significant (Table 2).
There was a significant decrease in plasma level of albumin ($p \leq 0.05$) in the salt-loaded rats compared to control, extract-treated normotensive, pre-treated salt-loaded, and salt-loaded rats treated with extract and standard drugs.
There was a decrease in the total protein level of the salt-loaded rats compared to the control, extract-treated normotensive, pre-treated salt-loaded, and salt-loaded rats treated with extract and captopril but was not statistically significant ($p > 0.05$).

The total protein level of the salt-loaded rats was significantly decreased ($p \leq 0.05$) compared to the salt-loaded rats treated with HCTZ as shown in Table 2.

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Table 1: Dose dependent effects of aqueous extract of *Emilia Praetermissa* leaf on SBP, DBP, MAP and AHR of hypertensive rats

GROUP	SBP			DBP		
	0h	1h	3h	0h	1h	3h
50 mg extract/kg	145.60 ± 2.13	123.20 ± 2.68*	99.67 ± 0.68*	113.60 ± 5.48	88.39 ± 2.76*	80.28 ± 2.20*
100 mg extract/kg	144.30 ± 2.07	95.11 ± 1.90*#	87.39 ± 1.34*#	111.60± 4.86	74.78 ± 3.11*#	70.11 ± 2.77*#
200 mg extract/kg	147.40 ± 1.62	124.00 ± 1.89*a	112.00 ± 0.86*#	114.20± 3.97	99.95 ± 2.32*#	88.22 ± 3.54*a
	MAP			**AHR**		
	0h	1h	3h	0h	1h	3h
50 mg extract/kg	124.10 ± 3.97	99.83 ± 1.68*	86.61 ± 1.42*	342.80 ± 10.76	314.90 ± 7.23*	316.10 ± 5.24a
100 mg extract/kg	122.30 ± 3.26	81.39 ± 2.58*#	75.67 ± 1.99*#	323.90 ± 10.46	301.00 ± 4.99aa	312.70 ± 3.49aa
200 mg extract/kg	125.00 ± 2.79	107.80 ± 1.80*#	96.03 ± 2.56*#	337.10 ± 10.88	325.30 ± 7.12aa	313.40 ± 9.91aa

SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), MAP (Mean Arterial Pressure), AHR (Average Heart Rate). Values are expressed as Mean ± SEM; Values with asterisk (*) mean significant in comparison with baseline values (0); Values with a hash symbol (#) mean significant in comparison with 50 mg extract/kg *Emilia praetermissa;* a= non-significant

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Table 2: Effects of aqueous extract of *Emilia Praetermissa* leaf on ALT, AST, ALP, GGT, albumin, total protein, and total bilirubin in plasma of hypertensive rats

Groups/Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	Albumin (g/dL)	Total protein (g/dL)	Total bilirubin (mg/dL)
I: Control	24.02±0.01[b]	43.18±0.01[b]	9.63±0.01[b]	0.64±0.01[b]	7.65 ± 0.01[b]	14.05 ± 0.25[a]	0.068 ± 0.00[a]
II: 8% NaCl	52.67 ± 0.18	46.51 ± 0.01	8.54 ± 0.01	0.87 ± 0.01	5.46 ± 0.01	13.74 ± 0.49	0.075 ± 0.00
III: *Ep* (100 mg/kg)	24.14 ± 0.01[b]	43.23 ± 0.01[b]	9.67 ± 0.01[b]	0.66 ± 0.01[b]	8.33 ± 0.01[b]	14.47 ± 0.25[a]	0.070 ± 0.00[a]
IV: *Ep* (100 mg/kg) + 8% NaCl	28.50 ± 0.02[b]	43.30 ± 0.00[b]	8.85 ± 0.01[a]	0.66 ± 0.01[b]	5.85 ± 0.01[b]	13.51 ± 0.45[a]	0.067 ± 0.00[a]
V: 8% NaCl + *Ep* (100 mg/kg)	31.50 ± 0.06[b]	43.36 ± 0.02[b]	8.86 ± 0.01[a]	0.73 ± 0.01[b]	5.86 ± 0.01[b]	14.40 ± 0.82[a]	0.073 ± 0.01[a]
VI: 8% NaCl + Captopril (50 mg/kg)	25.32 ± 0.01[b]	43.26 ± 0.01[b]	9.33 ± 0.01[b]	0.64 ± 0.00[b]	6.95 ± 0.02[b]	14.84 ± 0.49[a]	0.072 ± 0.00[a]
VII: 8% NaCl + HCTZ (10 mg/kg)	25.12 ± 0.01[b]	43.23 ± 0.01[b]	9.40 ± 0.01[b]	0.64 ± 0.01[b]	6.86 ± 0.01[b]	15.68 ± 0.52[b]	0.073 ± 0.01[a]

Ep (*Emilia Praetermissa*), NaCl (Sodium Chloride), HCTZ (Hydrochlorothiazide), ALT (Alanine transaminase), AST (Aspartate transaminase), ALP (Alkaline phosphatase), and GGT (Gamma glutamyl transferase). Values are expressed as mean ± SEM, n=6. Values with a superscript 'a' are not statistically significant while values with superscript 'b' are statistically significant ($p \leq 0.05$) relative to the untreated hypertensive group of rats.

Liver enzymes are commonly used as indicators of liver damage, as they are predominantly found within hepatocytes. When the hepatocyte membranes are compromised, these enzymes can leak into the bloodstream. [56] This study also examined the effects of salt loading on liver marker enzymes (ALT, AST, and ɤ-GT) in rat plasma, which suggested possible hepatic injury. However, treatment with the extract led to a reduction in these enzyme levels, indicating its protective role against salt-induced liver damage. These findings are comparable to the effects observed with the Roselle-Olive combination on ALT, AST, and γ-GT levels in L-NAME-induced hypertensive rats. [57] Furthermore, the extract reversed the abnormal levels of total protein, albumin, and total bilirubin seen in salt-loaded rats. The extract showed hepatoprotective potential, both when administered alone and in combination with NaCl, as it reduced the expression of the liver enzymes. Its effectiveness was comparable to standard treatments such as captopril and HCTZ in preserving liver function. These findings are comparable to the effects of the aqueous extract of *Tridax procumbens* leaf on plasma total protein, albumin, and total bilirubin in L-NAME-induced hypertensive rats. [58]

Effect of Emilia praetermissa on renal function status in normotensive and hypertensive rats

Plasma electrolytes, namely sodium, potassium, chloride, and bicarbonate levels were also determined. Plasma sodium and chloride levels were significantly increased ($p \leq 0.05$) in the salt-loaded rats compared to control, extract-treated normotensive, pre-treated salt-loaded, and salt-loaded rats treated with extract and standard drugs respectively (Table 3).

HCO_3^- ion was significantly decreased ($p \leq 0.05$) in the salt-loaded groups compared to control, extract-treated normotensive, pre-treated salt-loaded, and salt-loaded rats treated with extract and standard drugs respectively (Table 3).

Table 3: Effects of aqueous extract of *Emilia Praetermissa* Leaf on kidney function indices in hypertensive rats

Groups/Treatment	Urea (mg/dL)	Creatinine (mg/dL)	Na^+ (mmole/L)	K^+ (mmole/L)	Cl^- (mmole/L)	HCO_3^- (mmole/L)
I: Control	23.11 ± 0.01[b]	0.36 ± 0.01[b]	98.65 ± 0.01[b]	24.13 ± 0.01[b]	72.26 ± 0.01[b]	11.36 ± 0.01[b]
II: 8% NaCl	31.25 ± 0.01	0.46 ± 0.01	123.60 ± 1.18	26.44 ± 0.02	86.25 ± 0.01	9.14 ± 0.01
III: *Ep* (100 mg/kg)	23.09 ± 0.01[b]	0.32 ± 0.01[b]	98.83 ± 0.01[b]	24.41 ± 0.03[b]	72.31 ± 0.01[b]	9.53 ± 0.13[b]
IV: *Ep* (100 mg/kg) + 8% NaCl	25.51 ± 0.01[b]	0.33 ± 0.01[b]	98.83 ± 0.00[b]	31.05 ± 0.01[b]	72.42 ± 0.01[b]	10.36 ± 0.01[b]
V: 8% NaCl + *Ep* (100 mg/kg)	26.69 ± 0.09[b]	0.34 ± 0.00[b]	98.85 ± 0.01[b]	31.08 ± 0.01[b]	72.48 ± 0.01[b]	10.45 ± 0.03[b]
VI: 8% NaCl + Captopril (50 mg/kg)	24.27 ± 0.03[b]	0.35 ± 0.01[b]	98.56 ± 0.01[b]	31.10 ± 0.00[b]	72.36 ± 0.01[b]	10.86 ± 0.01[b]
VII: 8% NaCl + HCTZ (10 mg/kg)	25.03 ± 0.01[b]	0.33 ± 0.01[b]	98.54 ± 0.01[b]	31.11 ± 0.01[b]	72.29 ± 0.01[b]	10.87 ± 0.01[b]

Ep (*Emilia Praetermissa*), NaCl (Sodium Chloride), HCTZ (Hydrochlorothiazide), Na^+ (Sodium ion), K^+ (Potassium ion), Cl^- (Chloride ion), and HCO_3^- (Bicarbonate ion). Values are expressed as mean ± SEM, n=6. Values with a superscript 'a' are not statistically significant while values with superscript 'b' are statistically significant ($p \leq 0.05$) relative to the untreated hypertensive group of rats.

Plasma potassium was significantly increased ($p \leq 0.05$) in pre-treated salt-loaded and salt-loaded rats that were treated with extract and standard drugs compared to the salt-loaded, control, and extract-treated normotensive rats (Table 3).

Plasma urea and creatinine levels were significantly increased ($p \leq 0.05$) in the salt-loaded rats compared to control, extract-treated normotensive, pre-treated salt-loaded, and the salt-loaded rats treated with extract and standard drugs respectively as shown in Table 3.

Water and electrolyte homeostasis, including the transport and excretion of sodium, potassium, and chloride, are crucial for renal function. [59] Previous studies have shown that potassium supplementation effectively ameliorates high blood pressure and renal injury in spontaneously hypertensive rats (SHR). [60,61] Similar to the captopril/HCTZ used in this study, the extract increased plasma potassium levels, suggesting it may mitigate salt-induced nephron damage. This finding aligns with the effects observed with the hydro-ethanol extract of *Plantago asiatica* seeds in the management of SHR. [55] Plasma creatinine and blood urea levels are key biomarkers for assessing renal function and diagnosing kidney impairment or damage. [62] In this study, administration of the extract reduced the expression of creatinine and urea levels in salt-loaded rats, suggesting a potential nephroprotective effect. These findings are similar to the effects of the Roselle-Olive combination on creatinine and blood urea levels in L-NAME-induced hypertensive rats. [57]

High salt intake can disrupt the body's water and electrolyte homeostasis, including the regulation of sodium, potassium, and chloride, essential for proper kidney function. [63,64] Salt-loaded rats exhibited increased plasma sodium ion levels compared to control, in line with previous research showing elevated sodium levels due to salt loading. [65,66] Notably, administration of the extract normalized the elevated plasma chloride ion levels in salt-loaded rats, possibly by reducing plasma sodium levels and promoting chloride excretion. [66] This simultaneous reduction of sodium and chloride ions is consistent with the actions of antihypertensive agents, particularly diuretics. [67] This finding mirrors the effects observed with the aqueous extract of *Tridax procumbens* Linn [68] and *Viscum album* leaves on plasma sodium and chloride concentrations in salt-loaded rats. [69] Furthermore, salt-loaded rats showed lower plasma bicarbonate levels compared to controls, but extract treatment raised bicarbonate levels, likely due to enhanced chloride ion excretion. These findings suggest the extract may have nephroprotective properties. This effect is comparable to the impact of the aqueous extract of *Viscum album* leaf on bicarbonate ion concentration in salt-loaded rats. [69]

Effect of Emilia praetermissa on lipid profile in normotensive and hypertensive rats

In this study, there were significant increases ($p \leq 0.05$) in total cholesterol, low-density lipoprotein concentration and a significant decrease ($p \leq 0.05$) in high-density lipoprotein concentration in the salt-loaded rats compared to control, extract treated normotensive, pre-treated salt-loaded, and the salt-loaded rats treated with extract and standard drugs. Triacylglycerides and very low-density lipoprotein concentration were significantly ($p \leq 0.05$) decreased in plasma of salt-loaded rats compared to control, pre-treated salt-loaded, and the salt-loaded rats treated with extract and standard drugs. However, these decreases were not statistically significant ($p > 0.05$) when compared to extract-treated normotensive rats as shown in Table 4.

Table 4: Effects of aqueous extract of *Emilia Praetermissa* leaf on lipid profile of hypertensive rats

Groups/Treatment	Total Cholesterol (mg/dL)	Triacylglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
I: Control	65.19 ± 0.07[b]	195.40 ± 0.01[b]	11.27 ± 0.01[b]	37.39 ± 0.07[b]	39.08 ± 0.00[b]
II: 8% NaCl	94.29 ± 0.01	192.10 ± 0.01	10.06 ± 0.01	65.91 ± 0.01	38.43 ± 0.00
III: *Ep* (100 mg/kg)	63.29 ± 0.57[b]	192.40 ± 0.02[a]	18.14 ± 0.01[b]	42.78 ± 0.52 [b]	38.47 ± 0.00[a]
IV: *Ep* (100 mg/kg) + 8% NaCl	65.37 ± 0.02[b]	196.80 ± 0.06[b]	15.27 ± 0.01[b]	41.27 ± 0.02[b]	39.37 ± 0.01[b]
V: 8% NaCl + *Ep* (100 mg/kg)	68.51 ± 0.01[b]	195.60 ± 0.19[b]	13.51 ± 0.01[b]	42.90 ± 0.03[b]	39.12 ± 0.04[b]
VI: 8% NaCl + Captopril (50 mg/kg)	65.25 ± 0.01[b]	196.60 ± 0.01[b]	15.83 ± 0.01[b]	41.76 ± 0.01[b]	39.31 ± 0.00[b]
VII: 8% NaCl + HCTZ (10 mg/kg)	65.27 ± 0.01[b]	196.30 ± 0.01[b]	15.81 ± 0.01[b]	41.82 ± 0.01[b]	39.26 ± 0.00[b]

Ep (*Emilia Praetermissa*), NaCl (Sodium Chloride), HCTZ (Hydrochlorothiazide), HDL-C (High-density Lipoprotein-Cholesterol), LDL-C (Low-density Lipoprotein-Cholesterol), VLDL-C (Very low-density Lipoprotein-Cholesterol). Values are expressed as mean ± SEM, n=6. Values with a superscript 'a' are not statistically significant while values with superscript 'b' are statistically significant ($p \leq 0.05$) relative to the untreated hypertensive group of rats.

Hypertension and dyslipidaemia are major risk factors for cardiovascular diseases (CVD), contributing to over 80% of deaths and disabilities worldwide. [70,71] Salt loading has been shown to induce oxidative stress and lipid peroxidation, leading to the accumulation of lipid peroxides and elevated blood lipid levels. It can also influence the expression and activity of proteins involved in lipid transport and metabolism, resulting in increased plasma lipid levels.[72]

In this study, salt-loaded rats exhibited dyslipidaemia, characterized by elevated plasma levels of total cholesterol and LDL-C, and a decrease in HDL-C compared to the control group. Remarkably, treatment with the extract significantly reversed the dyslipidemic profile of these rats, nearly restoring total cholesterol, LDL-C, and HDL-C levels to normal. These findings strongly suggest that the extract may exert a cardio-protective role in reducing dyslipidaemia caused by salt-induced hypertension. These findings are consistent with the effects of the Roselle-Olive combination and the aqueous extract of *Hibiscus sabdariffa* (Roselle) on total cholesterol, LDL-C, and HDL-C levels in hypertensive rats. [57,72] Interestingly, plasma levels of triacylglycerides (TAG) and very-low-density lipoprotein (VLDL-C) were lower in salt-loaded rats compared to controls, which may seem unusual as salt loading typically disrupts metabolic processes. However, the reduction in TAG levels could be attributed to a combination of increased lipolysis, reduced fatty acid synthesis, enhanced fatty acid oxidation, and altered VLDL-C secretion. These adaptations may represent the body's response to the metabolic stress of high salt intake, even though other lipid markers such as cholesterol and LDL-C were negatively impacted. Similarly, the slight reduction in VLDL-C levels could be attributed to one or a combination of factors, including altered hepatic VLDL-C production, decreased TAG availability for VLDL-C synthesis, enhanced fatty acid oxidation and compensatory metabolic adjustments to salt loading. [73]

The analysis of lipid profiles shows that salt loading significantly disrupts lipid metabolism, leading to increased total cholesterol and LDL-C while decreasing HDL-C. The administration of the extract effectively reversed these effects, restoring total cholesterol, HDL-C, and LDL-C to near-normal levels. These findings suggest that the extract may offer protective benefits against dyslipidaemia associated with hypertension, much like standard antihypertensive medications such as captopril and HCTZ. This positions the extract as a potential alternative therapy for managing hypertension and associated cardiovascular risks.

Antioxidant activity of aqueous extract of Emilia Praetermissa leaf in normotensive and hypertensive rats

The effects of aqueous extract of *Emilia praetermissa* leaf, captopril, and HCTZ on cardiac MDA, SOD, CAT, GSH, and GPx in normotensive and hypertensive rats is presented in Table 5. There were marked increases ($p \leq 0.05$) in MDA levels in the cardiac tissue of salt-loaded rats compared to the control but marked decreases ($p \leq 0.05$) in MDA levels of the groups that received extract and standard drugs compared to the salt-loaded rats (Table 5). There were significant decreases ($p \leq 0.05$) in SOD activities in cardiac tissue of salt-loaded rats in comparison to control, but marked increases ($p \leq 0.05$) in SOD activities of groups that received extract and standard drugs compared to the salt-loaded rats (Table 5). There were significant decreases ($p \leq 0.05$) in CAT activities in cardiac tissue of salt-loaded rats compared to control, but significant increases ($p \leq 0.05$) in CAT activities of the groups that received extract and standard drugs compared to the salt-loaded rats (Table 5). There were significant decreases ($p \leq 0.05$) in GSH levels in heart tissue of salt-loaded rats in comparison to control, but marked increases ($p \leq 0.05$) in GSH levels of groups that received extract and standard drugs compared to the salt-loaded rats (Tables 5).

Table 5: Effects of aqueous extract of *Emilia Praetermissa* Leaf on cardiac tissue oxidative stress and antioxidant status

Groups/Treatment	MDA x10^{-3} mmole/mL	SOD (U/mL)	Catalase (U/mL)	Reduced glutathione (U/mL)	Glutathione peroxidase (U/mL)
I: Control	14.21 ± 0.01^b	1.48 ± 0.02^b	103.80 ± 0.18^b	2.14 ± 0.01^b	1.92 ± 0.01^b
II: 8% NaCl	30.14 ± 0.01	1.15 ± 0.01	95.91 ± 0.11	1.55 ± 0.01	1.62 ± 0.00
III: *Ep* (100 mg/kg)	13.02 ± 0.04^b	3.90 ± 0.23^b	116.40 ± 0.14^b	2.46 ± 0.02^b	2.13 ± 0.01^b
IV: *Ep* (100 mg/kg) + 8% NaCl	14.82 ± 0.11^b	3.18 ± 0.01^b	111.90 ± 0.31^b	2.26 ± 0.01^b	1.96 ± 0.01^b
V: 8% NaCl + *Ep* (100 mg/kg)	16.43 ± 0.04^b	3.45 ± 0.02^b	108.40 ± 0.16^b	2.33 ± 0.01^b	1.94 ± 0.01^b
VI: 8% NaCl + Captopril (50 mg/kg)	14.05 ± 0.03^b	3.13 ± 0.01^b	113.50 ± 0.08^b	2.250 ± 0.01^b	2.43 ± 0.01^b
VII: 8% NaCl + HCTZ (10 mg/kg)	16.28 ± 0.01^b	3.14 ± 0.01^b	113.50 ± 0.05^b	2.24 ± 0.01^b	2.42 ± 0.01^b

Ep (*Emilia Praetermissa*), NaCl (Sodium Chloride), HCTZ (Hydrochlorothiazide), SOD (Superoxide dismutase). Values are expressed as mean ± SEM, n=6. Values with a superscript 'a' are not statistically significant while values with superscript 'b' are statistically significant ($p \leq 0.05$) relative to the untreated hypertensive group of rats.

In the heart tissue, there were significant decreases ($p \leq 0.05$) in GPx activities of salt-loaded rats compared to control but significant increases ($p \leq 0.05$) in GPx activities of the groups that received extract and standard drugs compared to the salt-loaded rats (Table 5).

Excessive salt intake and the hypertension it induces, has been shown to negatively affect the endothelium, the inner lining of blood vessels, leading to endothelial dysfunction essential for maintaining blood vessel health and regulating blood pressure. This imbalance can trigger oxidative stress and inflammation in the blood vessels. [65] Our study highlights the detrimental effects of salt loading on oxidative stress, as evidenced by elevated malondialdehyde levels and increased oxidative stress in cardiac tissues of salt-loaded rats. We also observed a significant reduction in endogenous antioxidants, including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPx), in these rats.

The administration of the extract demonstrated effective mitigation of oxidative stress in the cardiac tissues of salt-loaded rats. It successfully restored the activities of antioxidant enzymes to near-normal levels. This protective effect is likely due to the extract's ability to scavenge free radicals and reactive oxygen species, thus combating oxidative stress. Treatment with *Emilia praetermissa* extract significantly reduced MDA levels and normalized antioxidant enzyme activities, showing effects comparable to standard antihypertensive drugs like captopril and HCTZ. The extract's ability to boost antioxidant enzyme activities suggests it has strong antioxidant properties, which may protect cardiac tissues from oxidative damage. These findings are similar to the effect of aqueous extract of *Hibiscus sabdariffa* (Roselle) on MDA, SOD, catalase, GSH and GPx, in salt-induced hypertension in Wistar rats. [72] This underscores the potential cardio-protective effects of *Emilia praetermissa* in managing salt-induced oxidative stress and related cardiovascular issues. In summary, the extract exhibits significant antioxidant potential, offering protection against salt-induced oxidative stress, a key factor in hypertension and cardiovascular diseases. This observation is expected since many plants from the Asteraceae family, including *E. praetermissa*, are known for their antioxidant properties, as demonstrated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. [74] The high tannin content in the extract may also contribute to reversing stress indices, as tannins can significantly restore antioxidant enzyme activities like SOD, CAT, GPx, and GSH, and improve the antioxidant state of the organs. [75]

Effects of Aqueous Extract of Emilia Praetermissa Leaf on Blood Pressure Parameters

The effects of aqueous extract of *Emilia praetermissa* leaf, captopril, and HCTZ on SBP, DBP, MAP, and HR in hypertensive rats were evaluated as shown in Table 6.

There were significant decreases ($p \leq 0.05$) in SBP and DBP of extract-treated normotensive, pre-treated salt-loaded, and salt-loaded rats treated with extract and standard drugs compared to control and salt-loaded rats (Table 6).

MAP was significantly ($p \leq 0.05$) decreased in extract-treated normotensive, salt-loaded rats treated with extract and standard drugs compared to control and salt-loaded rats but this decrease was not statistically significant ($p > 0.05$) in pre-treated salt-loaded rats when compared to the control and salt-loaded rats (Table 6).

Heart rates of extract-treated normotensive, salt-loaded rats treated with extract and HCTZ were decreased but they were not statistically significant ($p > 0.05$) when compared to control and salt-loaded rats. These decreases were significant ($p \leq 0.05$) in the pre-treated salt-loaded and salt-loaded rats treated with captopril when compared to control and salt-loaded rats (Table 6).

This study investigated the impact of salt-loading on normotensive rats, revealing significant increases in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) compared to controls. However, a notable reversal was observed in salt-loaded rats treated with the extract, where blood pressure levels significantly decreased. These findings are comparable to the effects of the aqueous extract of *Hibiscus sabdariffa* (Roselle) on SBP, DBP, MAP, and HR in salt-induced hypertension in Wistar rats. [72] This reduction in blood pressure might be due to a decrease in oxidative stress, as indicated by the improved plasma antioxidant status in these rats.[72]

Table 6: Effect of aqueous extract of *Emilia Praetermissa* Leaf on blood pressure parameters

Groups/Treatment	Average systolic blood pressure	Average diastolic blood pressure	Average mean arterial pressure	Average heart rate
I: Control	108.70 ± 4.54[b]	73.75 ± 3.93[b]	85.25 ± 3.98[b]	339.70 ± 11.95[a]
II: 8% NaCl	145.90 ± 1.95	101.40 ± 2.53	108.90 ± 1.92	339.50 ± 10.25
III: *Ep* (100 mg/kg)	88.72 ± 3.98[b]	61.97 ± 3.10[b]	70.67 ± 2.69[b]	309.80 ± 11.80[a]
IV: *Ep* (100 mg/kg) + 8% NaCl	125.30 ± 2.57[b]	83.72 ± 2.42[b]	97.43 ± 1.98[a]	274.30 ± 11.54[b]
V: 8% NaCl + *Ep* (100 mg/kg)	91.61 ± 3.37[b]	67.50 ± 2.67[b]	75.83 ± 2.76[b]	307.90 ± 10.95[a]
VI: 8% NaCl + Captopril (50 mg/kg)	97.95 ± 2.52[b]	66.39 ± 4.70[b]	76.67 ± 3.80[b]	284.90 ± 11.41[b]
VII: 8% NaCl + HCTZ (10 mg/kg)	98.69 ± 4.64[b]	73.16 ± 4.75[b]	81.45 ± 4.25[b]	327.40 ± 18.48[a]

Ep (*Emilia Praetermissa*), NaCl (Sodium Chloride), HCTZ (Hydrochlorothiazide). Values are expressed as mean ± SEM, n=6. Values with a superscript 'a' are not statistically significant while values with superscript 'b' are statistically significant ($p \leq 0.05$) relative to the untreated hypertensive group of rats.

The results suggest that the extract exhibits strong antihypertensive properties, comparable to standard treatments such as captopril and hydrochlorothiazide. *Emilia praetermissa* not only lowers blood pressure but also positively affects heart rate. These findings propose that *Emilia praetermissa* could be an effective natural option for managing hypertension, especially in cases related to salt-induced hypertension. Nonetheless, further research is needed to fully explore its effects on heart rate regulation, particularly in combination therapies.

Plasma levels of angiotensin II, aldosterone and 6-keto prostaglandin $F_{1\alpha}$ in normotensive and hypertensive rats.

Plasma levels of angiotensin II and aldosterone were markedly increased in salt-loaded rats ($p > 0.05$) when compared to control (Table 7). Reductions in Angiotensin II levels were observed in extract-treated normotensive, pre-treated salt-loaded, and salt-loaded rats treated with extract, captopril, and HCTZ but these reductions weren't statistically significant ($p > 0.05$) when compared to control and salt-loaded rats (Table 7). Levels of aldosterone in salt-loaded rats treated with extract, captopril, and HCTZ were markedly decreased but these decreases weren't statistically significant ($p > 0.05$) when compared to control and salt-loaded rats (Table 7). There were significant decreases ($p \leq 0.05$) in plasma aldosterone levels of extract-treated normotensive and pre-treated salt-loaded rats compared to control and salt-loaded rats (Table 7). Plasma level of 6-keto Prostaglandin $F_{1\alpha}$ was markedly decreased in salt-loaded rats but this decrease wasn't statistically significant ($p > 0.05$) when compared to control (Table 7).

Level of 6-keto Prostaglandin $F_{1\alpha}$ was significantly increased ($p \leq 0.05$) in salt-loaded rats treated with extract compared to control, salt-loaded rats and other groups (Table 7). Marked increases in plasma levels of 6-keto Prostaglandin $F_{1\alpha}$ were also observed in extract-treated normotensive, pre-treated salt-loaded, and salt-loaded rats treated with captopril and HCTZ but these increases were not statistically significant ($p > 0.05$) when compared to salt-loaded rats as shown in Table 7.

To gain further insight into the extract's mechanistic effects, the study assessed its impact on the renin-angiotensin system (RAS) in salt-loaded rats. These rats exhibited increased plasma levels of angiotensin II (Ang II). However, treatment with the extract brought these levels closer to those observed in the control group, indicating a potential inhibitory effect of the extract on the RAS in salt-induced hypertension. Angiotensin II (Ang II) is a key vasoconstrictor within the RAS and is crucial to the aetiology of hypertension. This has led to efforts to manage hypertension by targeting Ang II production, with many studies showing positive outcomes from inhibiting Ang II. [76,77]

Similarly, the study examined aldosterone (ALD) levels in salt-loaded rats, which were significantly elevated but normalized following extract treatment. ALD is an important component in the RAS, contributing to increased blood pressure. [78,79] The reduction in ALD levels after treatment suggests potential benefits for managing salt-induced hypertension.

Additionally, the study investigated plasma levels of 6-keto-$PGF_{1\alpha}$, a stable metabolite of PGI_2 known for its vasodilatory and anti-platelet aggregatory effects. In salt-loaded rats, plasma 6-keto-$PGF_{1\alpha}$ levels decreased, but this was reversed with extract administration. The increase in 6-keto-$PGF_{1\alpha}$ levels suggests a potential counteraction of the vasoconstrictive effects of salt-loading. Enhancing PGI_2 production has been associated with better hypertension control. [80] These findings are similar to the effects observed with the hydro-ethanol extract of *Plantago asiatica* seeds on serum Ang II and ALD levels in spontaneously hypertensive rats [55] and the results from Mao[81] on the hypotensive and angiotensin-converting enzyme inhibitory activities of *Eisenia fetida* extract in spontaneously hypertensive rats. Overall, the results indicate that the extract exhibits significant antihypertensive properties by inhibiting the RAAS (lowering angiotensin II and aldosterone) and promoting vasodilation (increasing prostacyclin production). These effects appear to be comparable to or even stronger than those of captopril and hydrochlorothiazide, suggesting that the extract could be a promising natural alternative for managing hypertension, particularly in sodium-sensitive individuals.

Table 7: Effects of aqueous extract of *Emilia Praetermissa* Leaf on plasma angiotensin II, aldosterone and 6-keto prostaglandin $F_{1\alpha}$ levels in hypertensive rats

Groups/Treatment	Angiotensin II (pmole/L)	Aldosterone (pg /mL)	6-keto prostaglandin $F_{1\alpha}$ (ng /L)
I: Control	462.40 ± 37.40[a]	639.10 ± 35.91[a]	163.40 ± 8.13[a]
II: 8% NaCl	489.10 ± 37.42	742.10 ± 42.59	148.40 ± 24.30
III: *Ep* (100 mg/kg)	433.40 ± 44.87[a]	622.50 ± 17.08[b]	165.70 ± 9.10[a]
IV: *Ep* (100 mg/kg) + 8% NaCl	397.30 ± 61.93[a]	593.40 ± 29.79[b]	179.90 ± 12.33[a]
V: 8% NaCl + *Ep* (100 mg/kg)	472.50 ± 42.21[a]	690.00 ± 18.80[a]	215.70 ± 21.02[b]
VI: 8% NaCl + Captopril (50 mg/kg)	437.20 ± 48.61[b]	691.10 ± 27.74	208.70 ± 18.07[a]
VII: 8% NaCl + HCTZ (10 mg/kg)	443.00 ± 61.33[a]	671.20 ± 30.11[a]	188.40 ± 20.19[a]

Ep (*Emilia Praetermissa*), NaCl (Sodium Chloride), HCTZ (Hydrochlorothiazide). Values are expressed as mean ± SEM, n=6. Values with a superscript 'a' are not statistically significant while values with superscript 'b' are statistically significant ($p \leq 0.05$) relative to the untreated hypertensive group of rats.

Effects of aqueous extract of Emilia Praetermissa leaf on heart, aorta and kidney ultrastructure of hypertensive rats

Histopathology of the heart

The histopathological evaluation of the hearts from different groups and the findings are evident in Plates 1(a), 1(b), 1(c), 1(d),1(e), 1(f), and 1(g). The heart of the normotensive group (control) showed a normal histological finding of the coronary artery and myocardial fibres with mild deposits of collagen (Plate 1a). The 8% NaCl group revealed a dilated coronary artery with thickened fibres surrounded by prominent collagen deposition with visible myocardial fibres and mild deposits of collagen (Plate 1b). Rats in the *Ep* (100 mg/kg) group revealed coronary artery with fibres surrounded by collagen deposition (blue) with visible myocardial fibres with a mild deposit of collagen (Plate 1c). Rats in the *Ep* (100 mg/kg) + 8% NaCl group reveal dilated congested coronary artery with thickened fibres (coloured red) surrounded by prominent collagen deposition (blue) with visible myocardial fibres with deposits of collagen (coloured blue) Plate1d. Rats in the 8% NaCl + *Ep* (100 mg/kg) group reveal coronary arteries with thickened fibres (coloured red) surrounded by mild collagen deposition (blue) with visible myocardial fibres with mild deposits of collagen coloured blue (Plate 1e). Rats in the 8% NaCl + captopril (50 mg/kg) group reveal dilated coronary artery with thickened fibres (coloured red) with visible myocardial fibres with almost no deposits of collagen (coloured blue) Plate 1f.

Rats in the 8% NaCl + HCTZ (10 mg/kg) group reveal coronary artery with fibres (coloured red) with visible myocardial fibres with mild deposits of collagen (coloured blue) Plate 1g.

In this study, the results demonstrate different levels of impact on the heart's ultrastructure based on treatment and salt intake. The normotensive group exhibits a normal cardiac structure, while salt intake leads to notable pathological changes, including dilation and collagen deposition. The extract provides partial protection but fails to fully counteract the damage induced by salt. In contrast, standard antihypertensive treatments like captopril and HCTZ show more effective management of collagen deposition and overall cardiac health. These findings are similar to the effects of the hydro-ethanol extract of *Plantago asiatica* seeds on heart tissues in spontaneously hypertensive rats. [55]

Histopathology of the aorta

The histopathological evaluation of the aorta of the different groups and findings are evident in Plates 2(a), 2(b), 2(c), 2(d), 2(e), 2(f) and 2(g).

The aorta of the normotensive group (control) showed a normal histological finding of the tunica intima with bundles of muscle layer; tunica media staining blue black and adventitia comprising of connective tissue staining magenta (Plate 2a). Rats in the 8% NaCl group reveal tunica intima with bundles of muscle layer; tunica media staining black indicative of increased deposition and adventitia comprising of connective tissue staining magenta (Plate 2b). Rats in the *Ep* (100 mg/kg) group reveal tunica intima, with bundles of muscle layer; tunica media with visible mononuclear and histiocytic infiltrates and adventitia comprising of connective tissue (Plate 2c). Rats in the *Ep* (100 mg/kg) + 8% NaCl group reveal tunica intima with bundles of muscle layer; tunica media staining black indicative of increased deposition and adventitia comprising of connective tissue staining magenta (Plate 2d). Rats in the 8% NaCl + *Ep* (100 mg/kg) group reveal tunica intima with bundles of muscle layer; tunica media staining black indicative of increased deposition and adventitia comprising of connective tissue staining magenta (Plate 2e). Rats in the 8% NaCl + captopril (50 mg/kg) group reveal tunica intima with bundles of muscle layer; tunica media staining black indicative of increased deposition and adventitia comprising of connective tissue staining magenta (Plate 2f). Rats in the 8% NaCl + HCTZ (10 mg/kg) group reveal tunica intima with bundles of muscle layer; tunica media staining blue black and adventitia comprising of connective tissue staining magenta (Plate 2g).

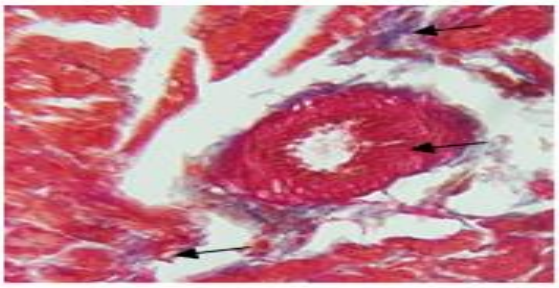

Plate 1a: Photomicrograph of a section of heart tissue from rats in control group (MT stain; x400).

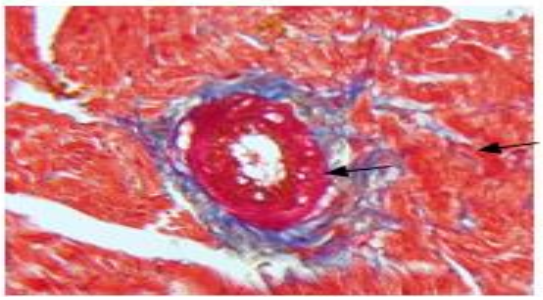

Plate 1b: Photomicrograph of a section of heart tissue from rats in the 8% NaCl group (MT stain; x400).

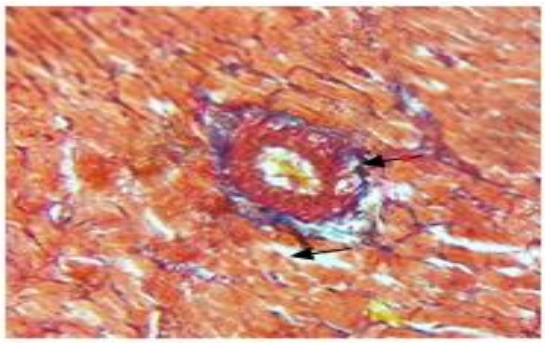

Plate 1c: Photomicrograph of a section of heart tissue from rats in the *Ep* (100 mg/kg) treated group (MT stain; x400).

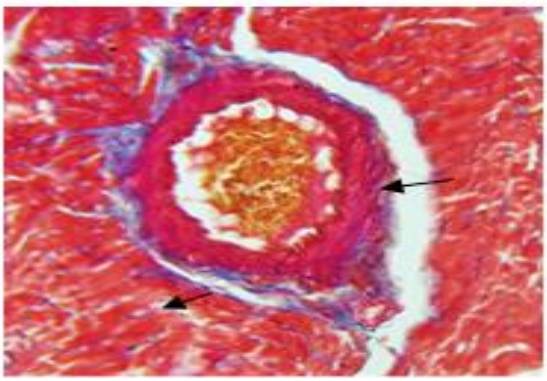

Plate 1d: Photomicrograph of a section of heart tissue from rats in the *Ep* (100 mg/kg) + 8% NaCl treated group (MT stain; x400).

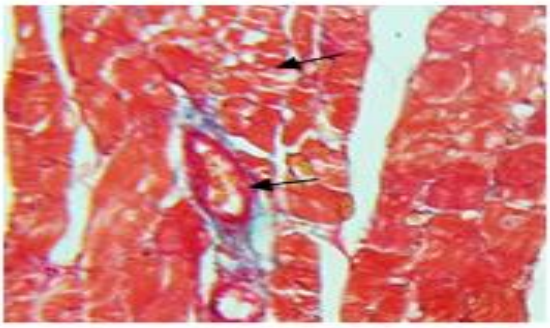

Plate 1e: Photomicrograph of a section of heart tissue from rats in the 8% NaCl + *Ep* (100 mg/kg) treated group (MT stain; x400).

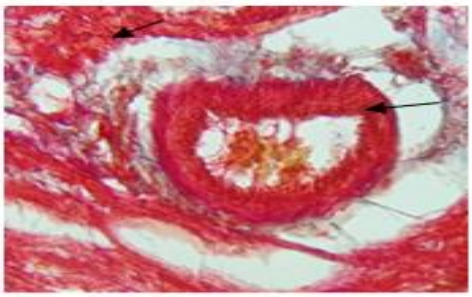

Plate 1f: Photomicrograph of a section of heart tissue from rats in the 8% NaCl + captopril (50 mg/kg) treated group (MT stain; x400).

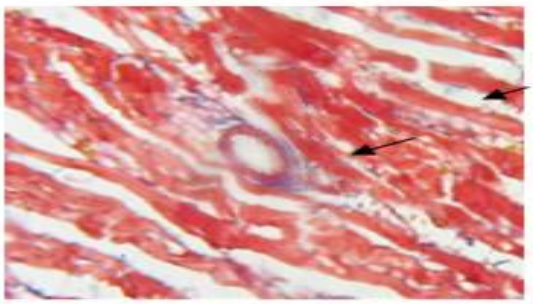

Plate 1g: Photomicrograph of a section of heart tissue from rats in the 8% NaCl + HCTZ (10 mg/kg) treated group. (MT stain; x400).

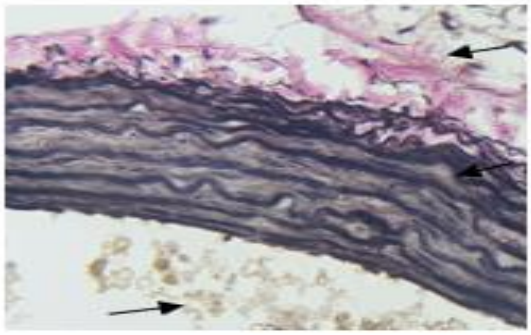

Plate 2a: Photomicrograph of a section of aorta from rats in control group (VVG stain; x400).

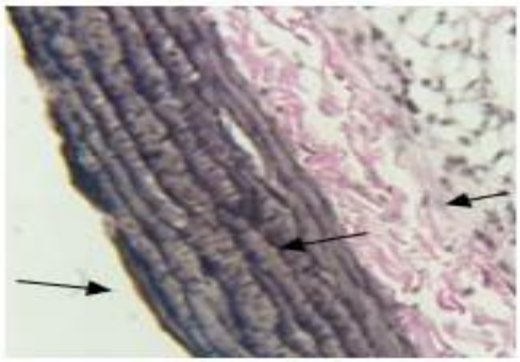

Plate 2b: Photomicrograph of a section of aorta from rats in the 8% NaCl group (VVG stain; x400).

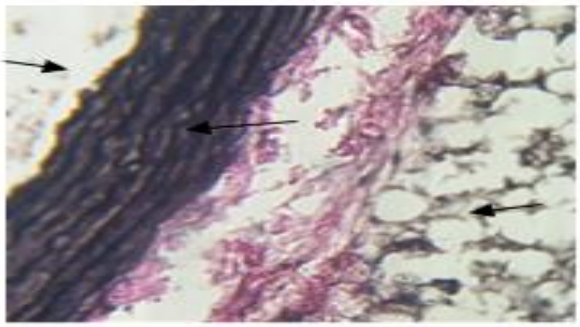

Plate 2c: Photomicrograph of a section of aorta from rats in the *Ep* (100 mg/kg) treated group (VVG stain; x400).

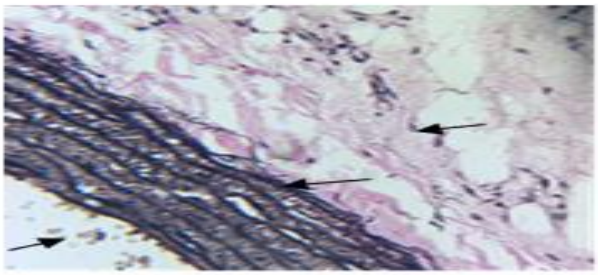

Plate 2d: Photomicrograph of a section of aorta from rats in the *Ep* (100 mg/kg) + 8% NaCl treated group (VVG stain; x400).

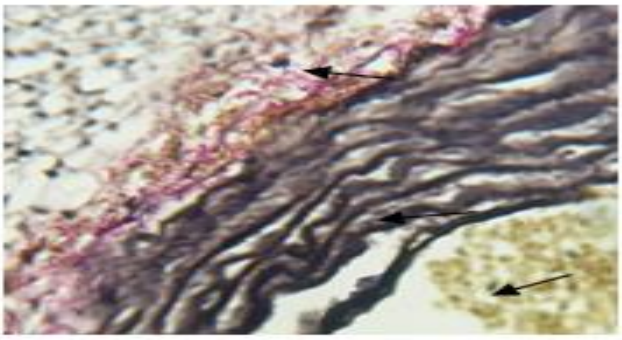

Plate 2e: Photomicrograph of a section of aorta from rats the 8% NaCl + *Ep* (100 mg/kg) treated group (VVG stain; x400).

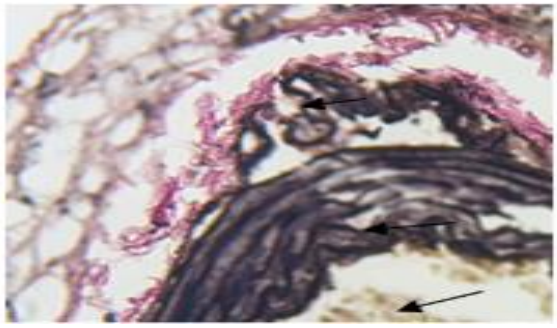

Plate 2f: Photomicrograph of a section of aorta from rats in the 8% NaCl + captopril (50 mg/kg) treated group (VVG stain; x400).

Plate 2g: Photomicrograph of a section of aorta from rats in the 8% NaCl + HCTZ (10 mg/kg) treated group. (VVG stain; x400).

The results from this study, highlight that salt intake induces significant pathological changes in the aorta, including increased deposition in the tunica media. The aqueous extract of *Emilia praetermissa* shows limited effectiveness in counteracting these changes. However, conventional antihypertensive treatments like captopril and HCTZ are more effective, with HCTZ demonstrating superior management of aortic structure by maintaining normal deposition levels. These findings are somewhat similar to the effects observed with the hydro-ethanol extract of *Plantago asiatica* seeds on aortic tissues in spontaneously hypertensive rats. [55]

Histopathology of the kidney

The histopathological evaluation of the kidney of the different groups and findings are evident in Plates 3(a), 3(b), 3(c), 3(d), 3(e), 3(f) and 3(g). The kidney of the normotensive group (control) showed a normal histological finding of renal corpuscles with magenta staining with prominent tubules and interstitial with blue/black nucleus (Plate 3a). Rats in the 8% NaCl group reveal renal corpuscles with deep magenta staining indicative of deposition of glomeruli casts with prominent tubules and interstitial cast deposition (magenta colour) with blue/black nucleus (Plate 3b). Rats in the *Ep* (100 mg/kg) group reveal renal corpuscles with deep magenta staining indicating a large deposition of glomeruli casts with prominent tubules and interstitial with blue/black nucleus (Plate 3c). Rats in the *Ep* (100 mg/kg) + 8% NaCl group reveal renal corpuscles with deep magenta staining indicative of deposition of glomeruli casts with prominent tubules and interstitial cast deposition (magenta colour) with blue/black nucleus (Plate 3d). Rats in the 8% NaCl + *Ep* (100 mg/kg) group reveal renal corpuscles with deep magenta staining indicative of deposition of glomeruli casts with prominent tubules and interstitial (magenta colour) with blue/black nucleus (Plate 3e). Rats in the 8% NaCl + captopril (50 mg/kg) group reveal renal corpuscles with deep magenta staining indicative of deposition of glomeruli casts with prominent tubules and interstitial (magenta colour) with blue/black nucleus (Plate 3f). Rats in the 8% NaCl + HCTZ (10 mg/kg) group reveal renal corpuscles with magenta staining with prominent tubules and interstitial with blue/black nucleus (Plate 3g).

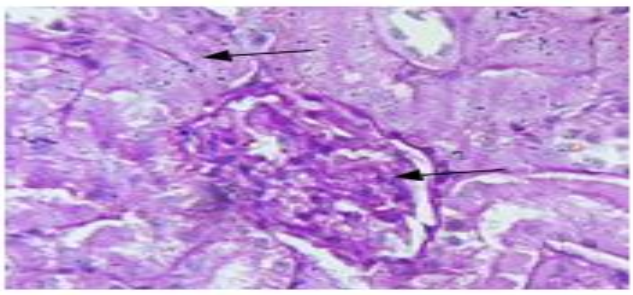

Plate 3a: Photomicrograph of kidney from rats in control group (PAS stain; x400).

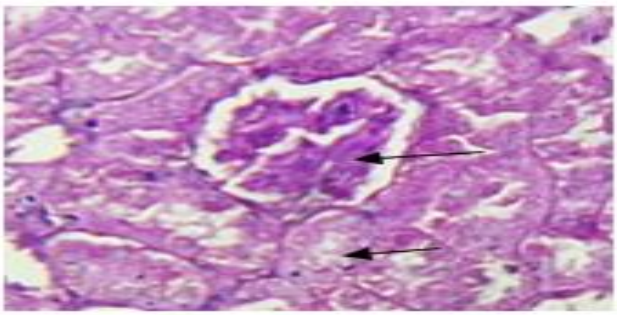

Plate 3b: Photomicrograph of kidney from rats in the 8% NaCl group (PAS stain; x400).

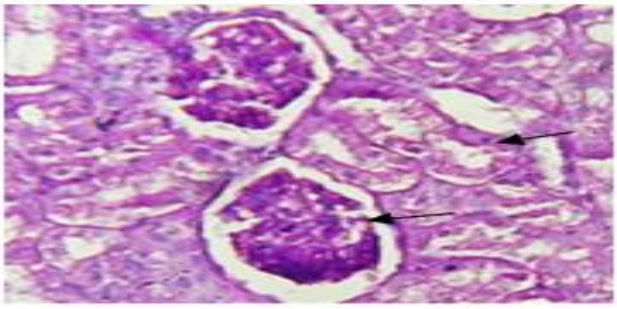

Plate 3c: Photomicrograph of kidney from rats in the *Ep* (100 mg/kg) treated group (PAS stain; x 400).

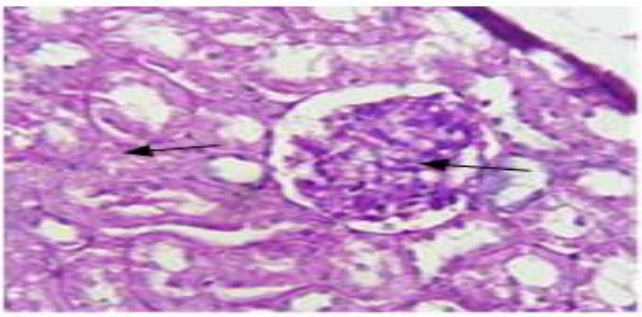

Plate 3d: Photomicrograph of kidney from rats in the *Ep* (100 mg/kg) + 8% NaCl treated group (PAS stain; x400).

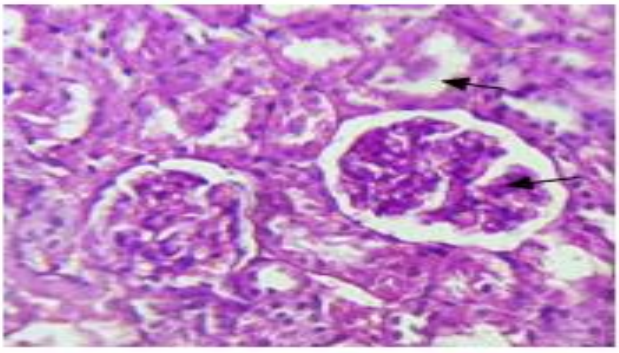

Plate 3e: Photomicrograph of kidney from rats in the 8% NaCl + *Ep* (100 mg/kg) treated group. (PAS stain; x400).

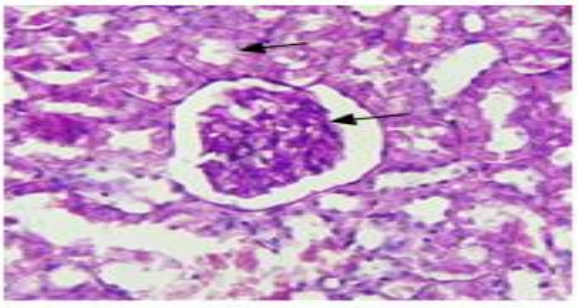

Plate 3f: Photomicrograph of kidney from rats in the 8% NaCl + captopril (50 mg/kg) treated group (PAS stain; x400).

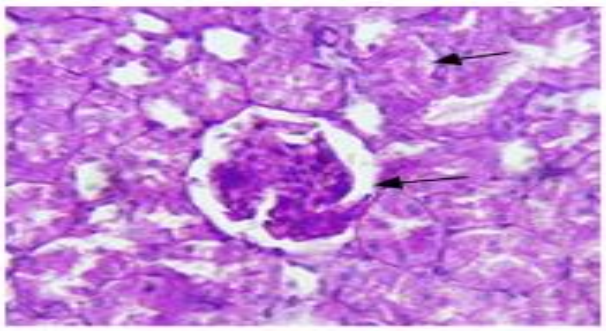

Plate 3g: Photomicrograph of kidney from rats in the 8% NaCl + HCTZ (10 mg/kg) treated group. (PAS stain; x400).

The results in this study, indicate that salt intake alone leads to substantial renal damage, characterized by glomeruli casts deposition and changes in kidney structure. This finding is consistent with the study by. [82] The extract showed limited effectiveness in preventing or reducing salt-induced renal damage, while captopril showed some degree of efficacy in reducing damage. In contrast, HCTZ was more effective in managing renal structure, offering the best protection among the treatments evaluated.

Histopathology of the liver

The histopathological evaluation of the liver of the different groups and findings are evident in Plates 4(a), 4(b), 4(c), 4(d), 4(e), 4(f) and 4(g). The liver of the normotensive group (control) showed a normal histological finding of prominent centriole with a spread of cytoplasm and keratin-coloured red. The hepatocytes reveal a nucleus that appears blue/black (Plate 4a).

Rats in the 8% NaCl group reveal prominent centriole with increased blue collagen deposition around the walls with a spread of cytoplasm and keratin-coloured red. The hepatocytes reveal a nucleus that appears blue/back (Plate 4b). Rats in the *Ep* (100 mg/kg) group reveal prominent centriole with blue collagen deposition around the walls with the spread of cytoplasm and keratin-coloured red. The hepatocytes reveal a nucleus that appears blue/black (Plate 4c). Rats in the *Ep* (100 mg/kg) + 8% NaCl group reveal a prominent centriole with increased blue collagen deposition around the walls with a spread of cytoplasm and keratin-coloured red. The hepatocytes reveal a nucleus that appears black (Plate 4d). Rats in the 8% NaCl + *Ep* (100 mg/kg) group reveal prominent centriole with mild blue collagen deposition around the walls with prominent spread of cytoplasm and keratin-coloured red. The hepatocytes reveal a nucleus that appears blue/black (Plate 4e). Rats in the 8% NaCl + captopril (50 mg/kg) group reveal prominent centriole with mild blue collagen deposition around the walls and spread of cytoplasm and keratin-coloured red. The hepatocytes reveal a nucleus that appears blue/black (Plate 4f). Rats in the 8% NaCl + HCTZ (10 mg/kg) group reveal visible centriole with a spread of cytoplasm and keratin coloured red. The hepatocytes reveal a nucleus that appears blue/black (Plate 4g).

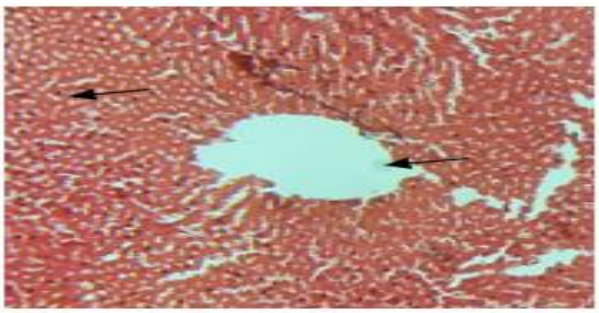

Plate 4a: Photomicrograph of liver from rats in control group (MT stain; x400).

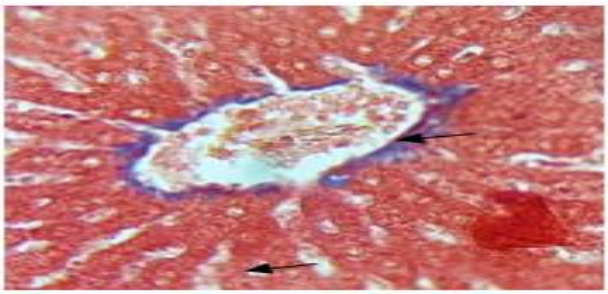

Plate 4b: Photomicrograph of liver from rats in the 8% NaCl group (MT stain; x400).

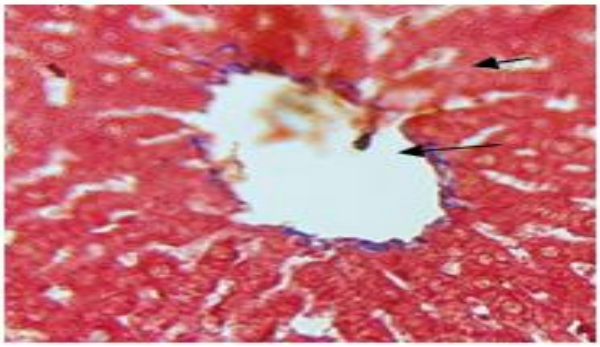

Plate 4c: Photomicrograph of liver from rats in the *Ep* (100 mg/kg) treated group. (MT stain; x400).

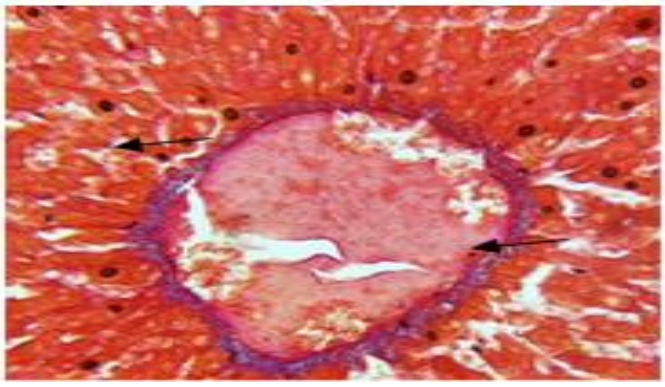

Plate 4d: Photomicrograph of liver from rats in the *Ep* (100 mg/kg) + 8% NaCl treated group (MT stain; x400).

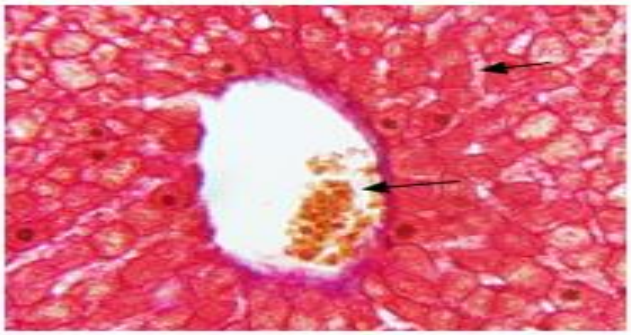

Plate 4e: Photomicrograph of liver from rats in the 8% NaCl + *Ep* (100 mg/kg) treated group. (MT stain; x400).

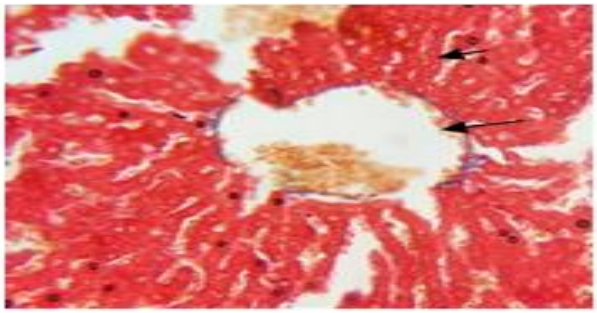

Plate 4.8f: Photomicrograph of liver from rats in the 8% NaCl + captopril (50 mg/kg) treated group (MT stain; x400).

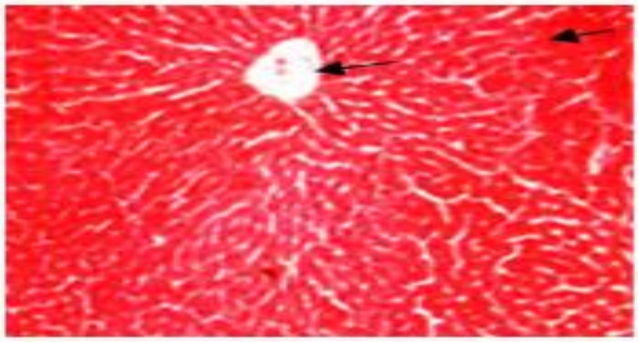

Plate 4.8g: Photomicrograph of liver from rats in the 8% NaCl + HCTZ (10 mg/kg) treated group. (MT stain; x400).

The results in this study, shows that while the extract provides some protection against liver damage, its efficacy is limited when compared to standard antihypertensive treatments. The extract did not fully mitigate the damage induced by salt intake, as indicated by persistent collagen deposition and alterations in hepatocyte appearance. Captopril offered some protection against liver damage, but HCTZ proved to be the most effective, demonstrating superior management of liver structure and offering the best protection among the treatments tested.

Conclusion

This study also adds credence to the claim that high salt intake causes hypertension and oxidative stress, evidenced by changes in antioxidant enzyme activities (SOD, CAT, GSH, and GPx). The aqueous extract of *Emilia praetermissa* leaf effectively reduced salt-induced hypertension, likely due to its ability to enhance antioxidant enzyme activities, lower angiotensin II and aldosterone levels, promote 6-keto-$PGF_{1\alpha}$ production, and improve liver and kidney function. While the extract provided partial protection to the heart, aorta, kidney, and liver, its effects were limited compared to standard antihypertensive treatments

like captopril and HCTZ. Salt intake caused significant pathological changes, including collagen deposition in the heart and aorta, with HCTZ offering the best protection. In the kidneys, salt-induced damage was minimal with the extract, while HCTZ provided the best protection. For the liver, the extract showed some protection, but captopril and HCTZ were more effective in preventing collagen deposition and hepatocyte damage.

Conflict of interest

The authors declare no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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