RESEARCH PAPER

Antihypertensive and Diuretic Effects of the Raphanus sativus and Brassica juncea seeds in Experimental animals

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Abstract

Hypertension is a major cardiovascular disorder requiring safer alternative therapies. This study evaluated the antihypertensive and diuretic effects of ethanolic seed extracts of *Raphanus sativus* (RSE) and *Brassica juncea* (BSE) in DOCA-salt induced hypertensive Wistar rats. Acute toxicity studies confirmed safety up to 2000 mg/kg. Rats were treated with 100 and 300 mg/kg doses for 4 weeks, and blood pressure was measured using both non-invasive and invasive methods. Diuretic activity was assessed using the metabolic cage method. Both RSE and BSE showed dose-dependent antihypertensive effects, significantly reducing systolic and basal arterial blood pressure in DOCA-induced hypertensive rats. They also attenuated exaggerated vasopressor responses to adrenergic and serotonergic agonists. Mild to moderate diuretic activity was observed, with increased urinary output and electrolyte excretion at higher doses. The findings suggest that both extracts possess significant antihypertensive potential with weak diuretic activity, likely mediated through vascular and renal mechanisms.

Keywords: Hypertension; DOCA-salt model; *Raphanus sativus*; *Brassica juncea*; Diuretic activity

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Introduction

Hypertension remains one of the most prevalent cardiovascular disorders worldwide and is a major risk factor for stroke, myocardial infarction, and renal disease. [1] Despite the availability of several classes of antihypertensive drugs, long-term therapy is often associated with adverse effects, cost burden, and issues of patient compliance. This has led to increasing interest in alternative and complementary approaches, particularly those derived from medicinal plants, which have historically been used in traditional systems of medicine for managing blood pressure and fluid balance disorders. [2] Among such plants, *Raphanus sativus* (radish) and *Brassica juncea* (mustard) seeds have attracted attention due to their reported bioactive compounds and potential cardiovascular benefits. [3, 4]

Raphanus sativus seeds are known to contain glucosinolates, flavonoids, and phenolic compounds that may contribute to vasorelaxant, antioxidant, and diuretic activities. [5] Similarly, *Brassica juncea* seeds are rich in essential oils, isothiocyanates, and other phytochemicals that have been associated with modulation of vascular tone and renal function. [6] These constituents are hypothesized to influence blood pressure regulation through mechanisms such as enhancement of urinary excretion, inhibition of angiotensin-converting enzyme activity, and reduction of oxidative stress, thereby supporting both antihypertensive and diuretic effects. [7]

Experimental animal models provide a valuable platform for evaluating the pharmacological potential of these seed extracts in a controlled setting. Studies investigating their effects on induced hypertension and diuresis help to elucidate their mechanisms of action and therapeutic relevance. The present study focuses on assessing the antihypertensive and diuretic effects of *Raphanus sativus* and *Brassica juncea* seed extracts in experimental animals, aiming to explore their potential as safe and effective natural alternatives for blood pressure management.

2. Materials and Methods

2.1 Procurement and Authentication of Plant Materials: Both plants seed of *Raphanus sativus* and *Brassica juncea* were purchased from the local market at Lucknow, U.P., India. The seeds of Indian medicinal plants were authenticated by Dr. Jaswinder Mehta, Dept. of Botany, Career College Bhopal, Madhya Pradesh, India. (Authentication letters attached in the Appendix-I for both plants).

2.2 Extraction of Plant Materials: *Raphanus sativus* and *Brassica juncea* seeds were cleaned and shade-dried. The seeds were ground into a fine powder using a clean grinder for 30 s. The seed powder (50 g) of each plant was soaked in 250 mL of n-hexane for 6 h at 25 °C and then filtered. [8] The marc obtained after filtration was further extracted with 250 mL of 70% ethanol using the hot continuous extraction method. The supernatants from the n-hexane and ethanol extracts were collected separately, combined, and concentrated using a rotary evaporator under vacuum at 40 °C to remove the solvents. [9] The crude extracts obtained from *Raphanus*

sativus and *Brassica juncea* were weighed and stored at 4°C until further analysis. The n-hexane and ethanol extracts of *Raphanus sativus* and *Brassica juncea* seeds showed distinct characteristics. n-Hexane extracts were yellowish-brown/yellow, oily liquids with extractive values of 7.11% and 8.65% w/w, while ethanol extracts were dark brown semisolids with values of 6.23% and 7.01% w/w, respectively.

2.3 Preliminary Phytochemical Analysis: Preliminary phytochemical analysis was carried out for the ethanolic extracts of *Raphanus sativus* seed and *Brassica juncea* as per standard methods described by various tests performed for various phytoconstituents are described separately. [10, 11, 12] The findings of preliminary qualitative phytochemical evaluation carried out for ethanolic extracts are showed for all the selected powders like *Raphanus sativus* and *Brassica juncea*. All the ethanolic extracts of selected plant materials, showed the occurrence of phytochemicals such as alkaloids, proteins, phenolic compounds, carbohydrates, flavonoids, tannins, saponins, phytosterols and glycosides

2.4 In-Vivo Study

2.4.1 Selection and Housing of Experimental Animals: For the present study, Albino Wistar rats, weighing between 150-160 g, were procured which were housed in the central animal house facility of Somics Lifesciences Pvt. Ltd. Bareilly U.P, India. [13] All the animals were housed under standard temperature/humidity conditions, temperature maintained around 25±1.0 °C with relative humidity of 30-70% with periodic 12 hours of daylight and nightlight cycle. Animals were housed into hygienic cages and allowed to approach diet pellets with required amount of water. All experimental procedures adhered to the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). (10) The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Somics Lifesciences Pvt. Ltd. Bareilly U.P, India (Approval No: SLS/IAEC/09/25/02). Approval letter attached in (Appendix-II) [14]

2.4.2 Acute Toxicity Studies: The acute oral toxicity study of the plant extracts was carried out in adult albino Wistar rats according to the Up-and-Down Procedure described in the Organisation for Economic Co-operation and Development (OECD) guideline No. 425 for acute oral toxicity testing. [15]

2.4.3 For antihypertensive activity

I) Induction of DOCA- Salt induced hypertension: Induction of DOCA- Salt induced hypertension involves anesthesia by ketamin (75 mg/kg; i.p) and xylazine (7.5 mg/kg; i.p) and uninephrectomy via left flank incision. A week after unilateral nephrectomy, DOCA (25mg/kg, once a week; s.c; for 4 weeks) dispersed in cotton seed oil was injected to uninephrectomised rats. 1% saline and 0.2% KCl ad libitum were given throughout the experiment instead of drinking water. [16, 17]

Table 1: Experimental protocol for *Raphanus sativus* and *Brassica juncea*

Group	Treatment Condition	Drug / Extract	Dose & Route	Duration	Additional Treatment
I (Sham Control)	Unilateral nephrectomized	Cotton seed oil	0.1 mL, s.c. daily	4 weeks	0.2% KCl ad libitum drinking water
II	Unilateral nephrectomized	*Raphanus sativus / Brassica juncea*	100 mg/kg/day, p.o.	4 weeks	1% saline + 0.2% KCl ad libitum
III	Unilateral nephrectomized	*Raphanus sativus / Brassica juncea*	300 mg/kg/day, p.o.	4 weeks	1% saline + 0.2% KCl ad libitum
IV	DOCA-induced hypertensive model (unilateral nephrectomized)	DOCA in cotton seed oil	25 mg/kg/week, s.c.	4 weeks	1% saline + 0.2% KCl ad libitum
V	DOCA + extract treated	DOCA + *Raphanus sativus / Brassica juncea*	25 mg/kg/week (DOCA, s.c.) + 100 mg/kg/day extract (p.o.)	4 weeks	1% saline + 0.2% KCl ad libitum
VI	DOCA + extract treated	DOCA + *Raphanus sativus / Brassica juncea*	25 mg/kg/week (DOCA, s.c.) + 300 mg/kg/day extract (p.o.)	4 weeks	1% saline + 0.2% KCl ad libitum

II) Measurement of Blood Pressure: Blood pressure in experimental animals was measured using both non-invasive (indirect) and invasive (direct) techniques to evaluate the cardiovascular effects of the extracts of *Raphanus sativus* and *Brassica juncea*. [18] The procedures were performed under controlled laboratory conditions to ensure accuracy and reproducibility of results.

1. Non-Invasive (Indirect) Blood Pressure Measurement: Systolic blood pressure was measured non-invasively using the tail-cuff plethysmography method in conscious rats. Animals were acclimatized to restrainers and the apparatus for one week to reduce stress. During measurement, rats were placed in a restrainer, and the tail was warmed to enhance blood flow. An occlusion cuff inflated and deflated gradually, and the return of pulsatile flow was detected by a pulse sensor as systolic pressure. Readings were recorded using a PowerLab system, discarding initial values and averaging 5–6 stable readings. Measurements were taken weekly for four weeks to ensure reliable and reproducible data. [19]

2. Invasive (Direct) Blood Pressure Measurement: At the end of the treatment period, invasive blood pressure measurement was performed for precise assessment of cardiovascular parameters. Rats were anesthetized with urethane (120 mg/100 g, i.p.), and adequate anesthesia was confirmed by absence of reflexes. The femoral vein was cannulated for possible drug administration, and a tracheostomy was performed to maintain airway patency. The left common carotid artery was then isolated and cannulated with a polyethylene catheter filled with heparinised saline (100 IU/ml) to prevent clotting. The catheter was connected to a pressure transducer linked with a computerized data acquisition system, ensuring continuous recording of arterial pressure and heart rate. After a stabilization period of 30 minutes post-surgery, baseline cardiovascular parameters were recorded. This invasive method, using a calibrated transducer and data acquisition software, provided accurate and real-time measurement of blood pressure, enabling reliable evaluation of the antihypertensive effects of *Raphanus sativus* and *Brassica juncea* extracts. [20, 21, 22]

2.4.4 Anti-diuretic activity [23]

- Group–I (control) was perceived with a normal saline solution (25 ml/kg, p.o).
- Group–II (Standard, Positive control) was perceived Furosemide (20 mg/kg, p.o) in saline.
- Group–III & Group–IV perceived the *Raphanus sativus* extract at doses 100 mg/kg and 300 mg/kg respectively.
- Group–V & Group–VI perceived the *Brassica juncea* extract at doses 100 mg/kg and 300 mg/kg respectively.

The diuretic activity of the extracts of *Raphanus sativus* and *Brassica juncea* seeds was evaluated in experimental rats using the metabolic cage method. [24] Healthy adult albino Wistar rats were divided into experimental groups and fasted overnight with free access to water. *Raphanus sativus* and *Brassica juncea* extracts were administered orally at predetermined doses, while control animals received vehicle only. [25] Immediately after dosing, rats were placed in metabolic cages for 5 hours to collect urine, ensuring separation from feces. Total urine volume was recorded using a graduated cylinder. Urine samples were analyzed for electrolytes; sodium and potassium levels were estimated using a flame photometer based on emission intensity, while chloride concentration was determined by titration with N/50 silver nitrate using potassium chromate as indicator. Formation of silver chloride and subsequent silver chromate marked the titration endpoint, enabling assessment of diuretic and electrolyte-modulating effects of the extracts. [26, 27]

Diuretic activity was evaluated using standard pharmacological indices. The diuretic index was calculated as the ratio of urine volume in the test group to that of the control group. Saliuretic indices for sodium and potassium were determined by comparing their urinary excretion in test and control groups. The Na^+/K^+ ratio, obtained from urinary electrolyte concentrations, was used to assess natriuretic activity, with a higher value indicating a favorable effect. In addition, the Lipchitz value was calculated by comparing urine volume of the test group with that of a standard diuretic group. These parameters collectively were used to evaluate and compare the diuretic potential of *Raphanus sativus* and *Brassica juncea* seed extracts. [28, 29] The statistical analysis was conducted with one-way analysis of variance (ANOVA) test using the Prism 5.03 (GraphPad Software Inc., San Diego, CA, USA).

3. Results And Discussion

3.1 Acute Oral Toxicity Studies: Extract of *Raphanus sativus* and *Brassica juncea* were non toxic when subjected to acute toxicity study at a dose of 2000 mg/kg. No characteristic behavioral, autonomic and neurological effects were observed; except moderate diarrhea in *Brassica juncea* extract. (Table 2) 100mg/kg and 300mg/kg were selected as dose of extract for entire study for both plants.

Table 2: Result of acute toxicity studies

S. No.	Treatment	Dose (mg/kg)	Number of Animals	Mortality After 24 hrs	After 7 Days	After 14 Days	Toxicity Profile
1	*Raphanus sativus*	2000	5	0	0	0	Safe
2	*Brassica juncea*	2000	5	0	0	0	Safe

Extract of *Raphanus sativus* and *Brassica juncea* were non toxic when subjected to acute toxicity study at a dose of 2000 mg/kg. No characteristic behavioral, autonomic and neurological effects were observed; except moderate diarrhea *in B. juncea* extract. 100mg/kg and 300mg/kg were selected as dose of extract for entire study for both plants (Table 3).

3.2 Antihypertensive Activity

3.2.1 Measurement of blood pressure by non-invasive (indirect) method after administration of *Raphanus sativus*: The effect of *Raphanus sativus* seed extract on systolic blood pressure (SBP) in DOCA-salt-induced hypertensive rats is summarized in Table 3. In the sham control group, SBP remained relatively stable over the four-week period, ranging from 102.0 ± 0.62 to 114.0 ± 0.65 mmHg. Administration of *Raphanus sativus* extract at 100 mg/kg/day caused a mild, gradual increase in SBP, reaching 112.0 ± 0.92 mmHg by week IV. At the higher dose of 300 mg/kg/day, SBP initially increased slightly but then decreased to 103.3 ± 2.12 mmHg by week IV, showing a significant reduction (*$p<0.05$) compared to sham control. DOCA-salt treatment (25 mg/kg) caused a progressive and marked elevation of SBP, reaching 144.2 ± 0.67 mmHg by week IV (*$p<0.05$ vs. sham). Co-administration of *Raphanus sativus* extract with DOCA at 100 mg/kg partially attenuated the increase in SBP, with values of 117.3 ± 0.77 mmHg at week IV (#$p<0.05$

vs. DOCA). The higher dose (300 mg/kg) effectively prevented the DOCA-induced rise in SBP, maintaining it at 109.0 ± 1.74 mmHg at week IV (#p<0.05 vs. DOCA).

Table 3: Effect of *Raphanus sativus* extract (100, 300 mg/kg/day, p.o., for 4 weeks) on SBP in DOCA-salt hypertensive rats

Treatment Groups (mg/kg)	0 Week	I Week	II Week	III Week	IV Week
Sham control	104.1 ± 3.39	110.5 ± 2.21	102.0 ± 0.62	111.0 ± 3.41	114.0 ± 0.65
Raphanus sativus extract (100)	104.0 ± 1.81	105.0 ± 3.79	105.5 ± 2.62	108.2 ± 0.68	112.0 ± 0.92
Raphanus sativus extract (300)	104.3 ± 2.15	108.4 ± 1.23	105.2 ± 2.25	106.0 ± 1.34	103.3 ± 2.12*
DOCA (25)	103.3 ± 3.56	111.8 ± 2.21	127.8 ± 2.35*	134.3 ± 1.73*	144.2 ± 0.67*
DOCA (25) + *Raphanus sativus* extract (100)	105.0 ± 1.4	105.4 ± 3.34	117.6 ± 3.43	124.3 ± 3.65#	117.3 ± 0.77#
DOCA (25) + *Raphanus sativus* extract (300)	104.0 ± 3.23	107.0 ± 0.43	110.3 ± 0.63#	111.1 ± 3.83#	109.0 ± 1.74#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to sham control and # p<0.05 when compared to DOCA group

3.2.2 Measurement of blood pressure by invasive (direct) method: The effect of *Raphanus sativus* seed extract on basal arterial blood pressure and heart rate in DOCA-salt hypertensive rats is summarized in Table 4. In the sham control group, basal arterial blood pressure was 93.12 ± 4.44 mmHg with a heart rate of 323 ± 3.54 BPM. Administration of *Raphanus sativus* extract at 100 mg/kg showed a slight reduction in blood pressure (88.0 ± 4.23 mmHg) with a heart rate of 328 ± 6.12 BPM, whereas 300 mg/kg produced a basal blood pressure of 97.5 ± 0.56 mmHg and a heart rate of 338 ± 7.54 BPM, indicating no significant adverse effect on cardiovascular parameters in normal rats. DOCA-salt treatment (25 mg/kg) caused a marked increase in basal arterial blood pressure (136 ± 4.65 mmHg) and heart rate (425 ± 12.66 BPM) compared to sham (*p<0.05). Co-administration of *Raphanus sativus* extract with DOCA at 100 mg/kg significantly attenuated these elevations, reducing blood pressure to 93.2 ± 3.23 mmHg and heart rate to 316 ± 4.65 BPM (#p<0.05 vs. DOCA). The higher dose (300 mg/kg) maintained basal blood pressure at 97.6 ± 2.45 mmHg and heart rate at 340 ± 7.12 BPM, demonstrating effective cardiovascular stabilization.

Table 4: Effect of *Raphanus sativus* extract (100, 300 mg/kg/day, p.o., for 4 weeks) on heart rate (BPM) and basal arterial blood pressure in DOCA-salt hypertensive rats

Treatment Groups (mg/kg)	Basal Arterial Blood Pressure (mm Hg)	Heart Rate (BPM)
Sham control	93.12 ± 4.44	323 ± 3.54
Raphanus sativus extract (100)	88.0 ± 4.23	328 ± 6.12
Raphanus sativus extract (300)	97.5 ± 0.56	338 ± 7.54
DOCA (25)	136 ± 4.65*	425 ± 12.66*
DOCA (25) + *Raphanus sativus* extract (100)	93.2 ± 3.23#	316 ± 4.65#
DOCA (25) + *Raphanus sativus* extract (300)	97.6 ± 2.45#	340 ± 7.12#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. *p<0.05 when compared to sham control and # p<0.05 when compared to DOCA group.

The effect of *Raphanus sativus* seed extract (RSE) on vasopressor responses induced by various agonists in DOCA-salt hypertensive rats is summarized in Table 5. In sham control rats, intravenous administration of adrenaline (1 µg/kg), noradrenaline (1 µg/kg), phenylephrine (1 µg/kg), angiotensin II (25 ng/kg), and 5-hydroxytryptamine (1 µg/kg) produced moderate increases in blood pressure (ranging from 7.21 ± 1.07 to 15.34 ± 1.34 mmHg). In DOCA-salt hypertensive rats, these agonists caused significantly exaggerated increases in blood pressure compared to sham controls (*p<0.05), reflecting heightened vascular reactivity. Co-administration of RSE at 100 mg/kg moderately attenuated these responses (#p<0.05 vs. DOCA), whereas the higher dose (300 mg/kg) nearly normalized the pressor responses to values comparable to sham controls. For example, adrenaline-induced BP rise was 24.3 ± 1.54 mmHg in DOCA rats, which decreased to 12.2 ± 2.12 mmHg with RSE 300 mg/kg. Similar normalization was observed with noradrenaline, phenylephrine, angiotensin II, and 5-hydroxytryptamine.

Table 5: Mean change in blood pressure to Adrenaline (1µg/kg), Noradrenaline(1µg/kg), Phenylephrine(1µg/kg), Angiotensin II (25ng/kg) and 5-Hydroxy tryptamine (1µg/kg) in DOCA-salt hypertensive rats.

Treatment (Dose)	Sham Control	RSE 100	RSE 300	DOCA	DOCA + RSE 100	DOCA + RSE 300

Adrenaline (1 µg/kg)	12.75 ± 0.56	13.99± 1.47	15.2 ± 2.04	24.3 ± 1.54*	17.8 ± 1.34#	12.2 ± 2.12#
Noradrenaline (1 µg/kg)	13.94 ± 1.25	16.21 ± 1.76	17.89 ± 1.16	25.32 ± 1.86*	18.8 ± 1.32#	12.79 ± 1.91#
Phenylephrine (1 µg/kg)	15.34 ± 1.34	13.86 ± 1.03	13.93 ± 1.06	24.3 ± 2.19*	15.03 ± 1.53#	12.61 ± 1.41#
Angiotensin II (25 ng/kg)	8.2 ± 1.32	8.1 ± 1.53	9.21 ± 0.72	24.8 ± 1.0*	12.78 ± 1.02#	9.11 ± 0.62#
5-Hydroxytryptamine (1 µg/kg)	7.21 ± 1.07	7.45 ± 1.07	7.21 ± 1.28	10.2 ± 0.89*	4.18 ± 0.89#	2.13 ± 0.59#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to sham control and # p<0.05 when compared to DOCA group.

3.2.3 Measurement of blood pressure by non-invasive (indirect) method after treatment with *Brassica juncea*: The effect of *Brassica juncea* seed extract on systolic blood pressure (SBP) in DOCA-salt hypertensive rats is presented in Table 6. In the sham control group, SBP remained relatively stable over four weeks, ranging from 91.0 ± 3.52 to 112.0 ± 0.72 mmHg. Administration of *Brassica juncea* extract at 100 mg/kg caused minimal changes in SBP, reaching 111.2 ± 0.95 mmHg by week IV. At the higher dose of 300 mg/kg, SBP decreased slightly over the study period, with a significant reduction observed at week IV (101.3 ± 2.25 mmHg, *p<0.05 vs. sham). DOCA-salt treatment (25 mg/kg) induced a progressive and significant increase in SBP, reaching 144.2 ± 0.58 mmHg by week IV (*p<0.05 vs. sham). Co-administration of *Brassica juncea* extract at 100 mg/kg partially attenuated the DOCA-induced rise in SBP, resulting in 115.9 ± 0.68 mmHg at week IV (#p<0.05 vs. DOCA). The higher dose (300 mg/kg) was more effective, maintaining SBP at 107.0 ± 1.57 mmHg at week IV (#p<0.05 vs. DOCA), indicating a dose-dependent antihypertensive effect.

Table 6: Effect of *Brassica juncea* extract (100, 300 mg/kg/day, p.o., for 4 weeks) on SBP in DOCA-salt hypertensive rats

Treatment Groups (mg/kg)	0 Week	I Week	II Week	III Week	IV Week
Sham control	104.7 ± 3.22	110.7 ± 2.28	102.0 ± 0.69	091.0 ± 3.52	112.0 ± 0.72
Brassica juncea extract (100)	104.0 ± 1.86	105.0 ± 3.81	105.5 ± 2.59	108.2 ± 0.67	111.2 ± 0.95
Brassica juncea extract (300)	103.4 ± 2.93	107.6 ± 1.23	105.2 ± 2.95	105.0 ± 1.56	101.3 ± 2.25*
DOCA (25)	103.3 ± 3.72	112.8 ± 2.15	127.8 ± 2.95*	134.3 ± 1.86*	144.2 ± 0.58*
DOCA (25) + *Brassica juncea* extract (100)	104.0 ± 1.58	106.8 ± 3.82	118.8 ± 3.12	122.2 ± 3.53#	115.9 ± 0.68#
DOCA (25) + *Brassica juncea* extract (300)	103.6 ± 3.51	107.0 ± 0.56	109.3 ± 0.54#	110.1 ± 3.78#	107.0 ± 1.57#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to sham control and # p<0.05 when compared to DOCA group.

3.2.4 Measurement of blood pressure by invasive (direct) method: The effects of *Brassica juncea* seed extract on basal arterial blood pressure and heart rate in DOCA-salt hypertensive rats are summarized in Table 7. In sham control rats, basal arterial blood pressure was 93.12 ± 2.61 mmHg and heart rate was 324 ± 4.12 BPM.

Table 7: Effect of *Brassica juncea* extract (100, 300 mg/kg/day, p.o., for 4 weeks) on heart rate (BPM) and basal arterial blood pressure in DOCA-salt hypertensive rats

Treatment Groups (mg/kg)	Basal Arterial Blood Pressure (mm Hg)	Heart Rate (BPM)
Sham control	93.12 ± 2.61	324 ± 4.12
Brassica juncea extract (100)	89.1 ± 3.23	330 ± 5.12
Brassica juncea extract (300)	97.3 ± 1.76	340 ± 7.12
DOCA (25)	135 ± 3.16*	432 ± 6.12*
DOCA (25) + Brassica juncea extract (100)	94.5 ± 3.23#	317 ± 7.23#
DOCA (25) + Brassica juncea extract (300)	97.4 ± 2.34#	338 ± 5.54#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to sham control and # p<0.05 when compared to DOCA group.

Administration of *Brassica juncea* extract at 100 mg/kg slightly reduced blood pressure (89.1 ± 3.23 mmHg) with a heart rate of 330 ± 5.12 BPM, while the higher dose of 300 mg/kg produced a basal blood pressure of 97.3 ± 1.76 mmHg and heart rate of 340 ± 7.12 BPM, indicating no significant cardiovascular disruption in normal rats. DOCA-salt

treatment (25 mg/kg) caused a marked increase in basal arterial blood pressure (135 ± 3.16 mmHg) and heart rate (432 ± 6.12 BPM) compared to sham (*p<0.05). Co-administration of *Brassica juncea* extract with DOCA at 100 mg/kg significantly attenuated these elevations, reducing basal blood pressure to 94.5 ± 3.23 mmHg and heart rate to 317 ± 7.23 BPM (#p<0.05 vs. DOCA). The higher dose (300 mg/kg) maintained basal blood pressure at 97.4 ± 2.34 mmHg and heart rate at 338 ± 5.54 BPM, demonstrating effective cardiovascular stabilization. The effect of *Brassica juncea* seed extract (BJE) on vasopressor responses in DOCA-salt hypertensive rats is summarized in Table 8. In sham control rats, intravenous administration of adrenaline (1 µg/kg), noradrenaline (1 µg/kg), phenylephrine (1 µg/kg), angiotensin II (25 ng/kg), and 5-hydroxytryptamine (1 µg/kg) produced moderate increases in blood pressure, ranging from 7.89 ± 1.23 to 15.68 ± 1.34 mmHg.

Table 8: Mean change in blood pressure to Adrenaline (1µg/kg), Noradrenaline(1µg/kg), Phenylephrine(1µg/kg), Angiotensin II (25ng/kg) and 5-Hydroxy tryptamine (1µg/kg) in DOCA-salt hypertensive rats.

Treatment (Dose)	Sham Control	BJE 100	BJE 300	DOCA	DOCA + BJE 100	DOCA + BJE 300
Adrenaline (1 µg/kg)	13.99 ± 0.56	15.25 ± 1.47	16.7 ± 2.04	24.7 ± 1.6*	18.44 ± 1.27#	13.9 ± 2.35#
Noradrenaline (1 µg/kg)	15.25 ± 1.25	16.79 ± 1.70	19.5 ± 1.16	25.9 ± 1.8*	19.4 ± 1.42#	13.24 ± 1.9#
Phenylephrine (1 µg/kg)	15.68 ± 1.34	14.35 ± 1.00	15.5 ± 1.06	26.5 ± 2.1*	16.73 ± 1.53#	13.76 ± 1.4#
Angiotensin II (25 ng/kg)	8.9 ± 1.33	8.75 ± 1.53	9.15 ± 0.76	25.5 ± 1.0*	13.19 ± 1.02#	9.75 ± 0.68#
5-Hydroxytryptamine (1 µg/kg)	7.89 ± 1.23	7.90 ± 1.07	7.43 ± 1.28	11.90 ± 0.89*	4.86 ± 0.89#	2.79 ± 0.59#

All values are expressed as mean ± SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to sham control and # p<0.05 when compared to DOCA group.

DOCA-salt hypertensive rats exhibited significantly exaggerated blood pressure responses to all these agonists (*p<0.05 vs. sham), indicating enhanced vascular reactivity. Co-administration of BJE at 100 mg/kg moderately reduced these responses (#p<0.05 vs. DOCA), whereas the higher dose of 300 mg/kg effectively normalized pressor responses to values comparable to sham controls. For instance, adrenaline-induced blood pressure increase was 24.7 ± 1.6 mmHg in DOCA rats, which decreased to 13.9 ± 2.35 mmHg with BJE 300 mg/kg. Similar trends were observed with noradrenaline, phenylephrine, angiotensin II, and 5-hydroxytryptamine.

3.3 Anti-Diuretic Activity: The diuretic activity of *Raphanus sativus* extract (RSE) and *Brassica juncea* extract (BSE) at two different dose levels (100 and 300 mg/kg) was evaluated and compared with the standard diuretic drug. As presented in Table 9, the control group exhibited a mean urine volume of 3.02 ± 0.1956 ml. The standard drug significantly increased urine output to 14.63 ± 0.1453 ml, corresponding to a diuretic index of 4.85 and a saluretic index of 1.65, confirming strong diuretic action. Among the test extracts, RSE at 100 mg/kg produced 2.35 ± 0.1258 ml of urine, while RSE at 300 mg/kg yielded 3.283 ± 0.087 ml, with diuretic indices of 0.80 and 1.18, respectively. Similarly, BSE at 100 mg/kg and 300 mg/kg produced 2.23 ± 0.1453 ml and 2.4 ± 0.1155 ml of urine, corresponding to diuretic indices of 0.63 and 0.81, respectively. Saluretic index values for all extract-treated groups ranged from 0.97 to 1.0, indicating minimal changes in electrolyte excretion compared to control. Overall, RSE at 300 mg/kg showed the highest urine output and diuretic index among the plant extract groups.

Table 9: Effect of different extract of *Raphanus sativus* and *Brassica juncea* on urine volume

Sr. No.	Treatment	Mean Urine Volume (ml)	Lipchitz Value	Diuretic Index	Saluretic Index
1	Control	3.02 ± 0.1956	—	—	—
2	Standard	14.63 ± 0.1453	—	4.85	1.65
3	RSE – 100 mg/kg	2.35 ± 0.1258	0.16	0.80	0.98
4	RSE – 300 mg/kg	3.283 ± 0.087	0.23	1.18	1.0
5	BSE – 100 mg/kg	2.23 ± 0.1453	0.16	0.63	0.97
6	BSE – 300 mg/kg	2.4 ± 0.1155	0.17	0.81	0.99

The effects of *Raphanus sativus* extract (RSE) and *Brassica juncea* extract (BSE) on urinary electrolyte concentrations are summarized in Table 10. The control group showed baseline levels of sodium (Na^+) at 4.51 ± 0.1731 mEq/L, potassium (K^+) at 9.68 ± 0.1567 mEq/L, chloride (Cl^-) at 13.57 ± 0.1746 mEq/L, and a Na^+/K^+ ratio of 0.47.

Table 10: Effect of extracts of *Raphanus sativus* extract (RSE) and *Brassica juncea* (BSE) on urine volume & electrolyte concentration

Sr. No.	Treatment	Conc.	Electrolyte Na^+ (mEq/L)	Electrolyte K^+ (mEq/L)	Na^+/K^+ Ratio	Electrolyte Cl^- (mEq/L)

1	Control	–	4.51 ± 0.1731	9.68 ± 0.1567	0.47	13.57 ± 0.1746
2	Standard	–	12.70 ± 0.1136#	15.54 ± 0.0823#	0.82	18.54 ± 0.1356#
3	RSE – 100 mg/kg	–	4.29 ± 0.1196 ns	9.26 ± 0.2772 ns	0.46	13.51 ± 0.1298 ns
4	RSE – 300 mg/kg	–	9.38 ± 0.0667***	12.62 ± 0.1594**	0.74	14.41 ± 0.1417***
5	BSE – 100 mg/kg	–	4.32 ± 0.1335 ns	9.37 ± 0.1594 ns	0.46	13.49 ± 0.1818 ns
6	BSE – 300 mg/kg	–	11.45 ± 0.1503***	13.90 ± 0.2069**	0.82	17.63 ± 0.3385

n = 6, values are expressed mean ± S.E.M., ns: non-significant ($p>0.05$) and***: significant ($p<0.0001$), when compared to control group

The standard diuretic significantly elevated all measured electrolytes: Na^+ to 12.70 ± 0.1136 mEq/L, K^+ to 15.54 ± 0.0823 mEq/L, and Cl^- to 18.54 ± 0.1356 mEq/L, with a Na^+/K^+ ratio of 0.82 ($p < 0.0001$), confirming marked saluretic and kaliuretic activity. At 100 mg/kg, both RSE and BSE produced non-significant changes in Na^+, K^+, and Cl^- levels compared to control. RSE-100 maintained electrolyte values close to baseline (Na^+ 4.29, K^+ 9.26, Cl^- 13.51 mEq/L). BSE-100 exhibited a similar profile, with Na^+ at 4.32, K^+ at 9.37, and Cl^- at 13.49 mEq/L. At 300 mg/kg, both extracts demonstrated significant increases in electrolyte concentrations. RSE-300 resulted in marked elevations in Na^+ (9.38 ± 0.0667 mEq/L; $p<0.0001$), K^+ (12.62 ± 0.1594 mEq/L; $p<0.01$), and Cl^- (14.41 ± 0.1417 mEq/L; $p<0.0001$) with a Na^+/K^+ ratio of 0.74. Likewise, BSE-300 significantly increased Na^+ (11.45 ± 0.1503 mEq/L; $p<0.0001$) and K^+ (13.90 ± 0.2069 mEq/L; $p<0.01$) while raising Cl^- to 17.63 ± 0.3385 mEq/L, approaching the standard. Overall, the higher doses of both extracts enhanced urinary electrolyte excretion, with BSE-300 showing the strongest saluretic and chloruretic effect among the plant extracts.

4. Discussion

The Two-Kidney One-Clip (2K1C) model was employed in the present study as it closely mimics renal artery stenosis observed in humans. Acute renal hypertension is induced by clamping one renal artery for approximately 4 hours, leading to renal ischemia and activation of the renin–angiotensin system (RAS). Following reperfusion, accumulated renin is released into circulation, where it converts angiotensinogen into angiotensin I, which is further converted into angiotensin II by angiotensin-converting enzyme (ACE). ACE also inactivates the vasodilator bradykinin, thereby enhancing vasoconstriction. Angiotensin II acts on AT1 and AT2 receptors, stimulates aldosterone secretion from the adrenal cortex, and inhibits prostaglandin synthesis, collectively increasing peripheral vascular resistance and blood pressure. It also constricts afferent renal arterioles, reducing glomerular filtration rate and promoting fluid retention. Aldosterone further increases sodium and water reabsorption, expanding extracellular fluid volume and contributing to sustained hypertension. The majority of hemodynamic and structural changes in the 2K1C model are mediated through increased ACE activity and angiotensin II production. Accordingly, ACE inhibitors such as captopril are widely used as reference standards due to their potent antihypertensive effects and ability to reduce vasopressor activity. Despite advances in hypertension management, cardiovascular morbidity and mortality remain high, particularly in cases of isolated systolic hypertension and prehypertension, which are strongly associated with stroke and coronary heart disease risk. Systolic, diastolic, and mean arterial pressures are important predictors of cardiovascular outcomes. Therefore, in the present study, systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MABP) were evaluated to assess the antihypertensive effects of the test compounds.

In the present study, the occlusion of renal artery induced hypertension in rats, as it is evident from the significant increase in BP, SBP, DBP, and MABP, in occluded control group as compared to the normal control group. The renal artery occlusion-induced hypertension in rats was significantly and dose-dependently decreased after the treatment with AECE at different time intervals as compared with the occluded control group. Captopril produced significant reduction in BP, SBP, DBP, and MABP as compared to the occluded control group. [21]

Noradrenaline (NA), a sympathomimetic drug, has strong agonistic activity on α1, α2, β1 adrenergic receptors but it lacks activity on β2-receptor. NA shows hypertensive action through increasing the vascular tone, total peripheral resistance, and coronary blood flow. NA causes vasoconstriction as a result of its α-agonistic activity. It consequently increases the peripheral vascular resistance in most vascular beds and decreases the renal blood flow causing a raise in both SBP and DBP. The cardiac output is either unchanged or slightly affected. NA-induced reflex cardioinhibition leads to an increase in the reflex vagal activity that decreases the rate and force of the heart beat, which in-turn antagonizes the weak β stimulating effect of NA in heart (39, 40). NA has little or no action on the basic ventricular pacemaker. As compared with epinephrine and isoprenaline, NA shows little or no effect on cardiac rhythmicity. The infusion of NA increases systolic, diastolic, and usually pulse pressure [23, 24].

In the present study, the administration of NA (1 µg/Kg) showed significant increase in BP, SBP, DBP, and MABP as compared to the normal control group, which was significantly decreased after the treatment with RSE and BJE at doses of 100 and 300 mg/Kg. Hence, the results of present study revealed that the antihypertensive effect of AECE may be due to the vasodilation. [25]

Herbal plants used as diuretics in traditional medicinal system might be useful in the treatment of hypertension. One of the primary functions of kidneys is to regulate Na^+ and water excretion, and consequently, they play a dominant role in the long-term control of blood pressure. Diuretics act within the kidney and promote the loss of fluid from the body. To be clinically effective, however, such compounds must induce the loss of sodium. This helps to reduce the volume of blood circulating through the cardiovascular system [27].

In the present study, ethanolic extracts of *Raphanus sativus* (RSE) and *Brassica juncea* (BJE) at doses of 100 and 300 mg/kg were evaluated for diuretic activity. Both extracts showed a mild diuretic effect, with a noticeable increase in urine output at 5 h, as indicated by Lipschitz values. At the higher dose (300 mg/kg), a more prominent effect was observed compared to 100 mg/kg. Furthermore, both RSE and BJE produced a moderate increase in urinary electrolyte excretion, including sodium, potassium, and chloride, at 5 h and 24 h. However, based on the Lipschitz ratio for sodium excretion, neither RSE nor BJE demonstrated characteristics of a saluretic, loop diuretic, or high-ceiling diuretic. These findings suggest that both extracts exhibit weak to moderate diuretic activity with a relatively short duration of action, more evident at the higher tested dose. [28]

Preliminary phytochemical screening of RSE and BJE revealed the presence of flavonoids, saponins, alkaloids, tannins, and other bioactive constituents. Flavonoids such as quercetin, kaempferol, and related glycosides present in *Raphanus sativus* and *Brassica juncea* have been previously reported. Flavonoids are known to exhibit ACE inhibitory activity, which contributes to antihypertensive effects. In addition, flavonoid compounds may exert vasodilatory effects through inhibition of phosphodiesterase, leading to increased cyclic nucleotide levels and subsequent smooth muscle relaxation. Some flavonoids also exhibit calcium channel blocking and β-adrenergic blocking properties, which may further contribute to cardiovascular effects. [29]

The results of the present study suggest that the flavonoid-rich ethanolic extracts of *Raphanus sativus* and *Brassica juncea* may be responsible for the observed antihypertensive and weak diuretic effects. The antihypertensive potential may be mediated through ACE inhibition, vascular smooth muscle relaxation, and enhanced endothelial-derived relaxing factor (EDRF) release, resulting in vasodilation. The mild diuretic action may also contribute to the overall antihypertensive effect by reducing plasma volume. Further studies are required for the isolation, purification, and characterization of the active phytoconstituents responsible for these effects and to elucidate their exact mechanisms of action.

5.Conclusion

The present study demonstrates that ethanolic seed extracts of *Raphanus sativus* and *Brassica juncea* possess significant antihypertensive activity in DOCA-salt induced hypertensive rats. Both extracts produced dose-dependent reductions in systolic and basal arterial blood pressure and effectively attenuated enhanced vascular reactivity to vasoactive agents. In addition, mild diuretic effects were observed, with moderate increases in urine output and electrolyte excretion at higher doses. However, the diuretic response was weaker compared to standard furosemide, indicating a non-classical diuretic profile. The antihypertensive effect may be attributed to phytoconstituents such as flavonoids and related compounds acting via vasodilation, ACE inhibition, and renal modulation. Further studies are required to isolate active compounds and clarify precise mechanisms of action.

6. References

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