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IMPACT OF ANTI-INFLAMMATORY AGENT LIKE *BRYOPHYLLUM PINNATUM* AQUEOUS EXTRACT AND DICLOFENAC ON BLOOD PRESSURE AND CREATININE CLEARANCE.

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ABSTRACT… Objectives: To study the impact of anti-inflammatory agent like *Bryophyllum pinnatum* aqueous extract and diclofenac on blood pressure and creatinine clearance. **Study Design:** Experimental study. **Setting:** Sargodha Medical College, Sargodha, Department of Pharmacy, University of Sargodha. **Period:** 1st January 2019 to 30th June 2019. **Material & Methods:** 24 Sprague Dawley rats were obtained and were then divided into four groups. Negative control group (A) contained animals received normal diet while Group B, C and D received diet containing sucrose (20% w/w) to induce hypertension. After that group B (positive control group) received distilled water 0.5 ml was by oral route, group C (*Bryophyllum pinnatum* group) received *Bryophyllum pinnatum* aqueous extract 300 mg/ml intraperitoneally and group D received diclofenac 12 mg/kg intraperitoneally as a single morning dose for two weeks. Blood pressure of animals was recorded at baseline and then weekly throughout the study using tail cuff using non-invasive blood pressure controller (ML125R). Animals were anesthetised with chloroform and two ml blood was drawn through cardiac puncture at 0, 4 and 6 weeks. Blood was tested for haematocrit. Serum sodium and potassium levels were estimated by flame photometer. Urinary creatinine levels were estimated by kinetic Jaffé method. The data collected was processed by using Statistical Package for Social Sciences (SPSS 20). **Results:** Mean and standard deviation of systolic blood pressure (BP) of group A rats did not change with time, while that of group B, C and D increased till 4th week. Creatinine clearance of group A, B and C did not change much with time but that of group D decreased towards end of study period. **Conclusion:** *Bryophyllum pinnatum* leaf aqueous extract is an effective anti-hypertensive agent with minimal renal effects.

Key words: Bryophyllum Pinnatum, Hypertension, Anti-inflammatory, Diclofenac.

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INTRODUCTION

Hypertension is a highly prevalent pathological condition.[1] It is considered as one of the most pertinent cause of cardiovascular accidents, imbalance of prostaglandins causes oxidative stress in tissues which eventually lead to hypertension in an individual.[2] *Bryophyllum pinnatum* is a small erect plant that belongs to family Crassulaceae (Milad et al., 2014). Its other common names are "pathar chat" in Urdu, air plant and life plant in English.[3]

Anti-inflammatory effects of *Bryophyllum pinnatum* are known for centuries.[4] It is used traditionally as hemostatic, given orally for urethritis, fever, arthritis, internal bruises, fractures, and respiratory inflammations and topical preparation for migraines and headaches.[5]

Analgesics commonly used in Pakistan include paracetamol, diclofenac and aspirin. Diclofenac has is an anti-inflammatory agent with a very pronounced analgesic effect.[6] Since a decade it is known that diclofenac blocks the arachidonic acid pathway as all other Non-steroidal anti-inflammatory agents. This pathway causes in reduction in formation of prostaglandins which evoke pain in an individual. In addition to its anti-inflammatory effect it has diverse effects on cardiovascular and renal system. These include elevated blood pressure, salt and water retention, clot formation and increased risk of cardiovascular

accidents like myocardial infarction and ischemic stroke.[7] In this regard, it is important to look for safer drugs that can effectively reduce the pain while sparing the vital organs of the body.

Both *Bryophyllum pinnatum* and diclofenac act via inhibition of arachidonic acid and result in reduced amount of prostaglandins and thromboxanes. In this study, we compared effect of both agents on blood pressure and renal function in a rodent model of hypertension.

MATERIAL & METHODS

Leaves of *Bryophyllum pinnatum* were collected and identified by the Botany Department of University of Sargodha, Sargodha. Leaves of *Bryophyllum pinnatum* were washed with tap water then dried under shade. They were crushed manually and immersed in distilled water in 1:10 (w/v) for 24 hours.[8] To filter supernatant, Whattman filter paper no.1 was used. The extract was dried by evaporation by putting in electrothermal heater. in order to avoid burning the heater temperature was set between 40 to 60 ◦C. The dry extract was weighed and kept at 4°C. *Bryophyllum pinnatum* aqueous extract containing 300 mg/ml was prepared daily using normal saline.[9]

24 Sprague Dawley rats were obtained and were kept in the Sargodha Medical College animal facility for 2 weeks for acclimatization. Room temperature was maintained at 25±5°C. Light dark cycle of 12 hrs was maintained and animals were given free access to food and tap water. They were then divided into four groups. Negative control group (A) contained animals received normal diet throughout the study period. Group B, C and D received diet containing sucrose(20% w/w)to induce hypertension.[10] By this method hypertension was induced in four weeks. After that group B (positive control group) received distilled water 0.5 ml was by oral route, group C (*Bryophyllum pinnatum* group) received *Bryophyllum pinnatum* aqueous extract 300 mg/ml intraperitoneally[9] and group D received diclofenac 12 mg/kg intraperitoneally as a single morning dose for two weeks.[11]

Blood pressure of animals was recorded at baseline and then weekly throughout the study. Five Systolic blood pressure readings were measured by tail cuff[12] using non-invasive blood pressure controller (ML125R) attached to computer based data recording system. Animals were anesthetised with chloroform and two ml blood was drawn through cardiac puncture at 0, 4 and 6 weeks. Blood was tested for hematocrit by haematology analyzer Sysmex Kx-21. Serum sodium and potassium levels were estimated by flame photometer. Creatinine estimation is gold standard for measuring renal function and kinetic Jaffé method was used method for this purpose.[13] Twenty four hour urine was collected at 0, 4 and 6 weeks by keeping animals in individual cages. Urinary creatinine levels were estimated by kinetic Jaffé method. Creatinine clearance was calculated using following formula.

$$\text{Clearance} = \frac{\text{Urine creatinine concentration x urine flow rate}}{\text{Plasma concentration}}$$

STATISTICAL ANALYSIS

The data collected was processed by using Statistical Package for Social Sciences (SPSS 20). It was checked for normal distribution by using Shapiro-wilk test of normality. Systolic blood pressure and creatinine clearance was presented as mean ± standard deviation (SD). One way ANOVA was used to test significance of difference between systolic blood pressure and creatinine clearance at 0, 4th and 6th week. Post hoc Tukey's test was used to analyse difference systolic blood pressure and creatinine clearance among groups A, B, C and D at 0, 4th and 6th week. p value of ≤ 0.05 was considered significant, ≤ 0.01 highly significant and ≤ 0.001very highly significant.

RESULTS

Mean and standard deviation of systolic blood pressure (BP) of all groups are given in table 1 below. BP of group A rats did not change with time, while that of group B, C and D increased till 4th week. ANOVA revealed that difference among groups was not significant at week 0, while it was highly significant at 4th and 6th week.

Post hoc Tukey's test was applied to compare

blood pressure among groups. It revealed that systolic blood pressure was significantly high in group B, C and D as compared to group A, where as they were reduced in group C and increased in group B at 6th week. Paired t-test was applied to compare changes in blood pressure within each group with time. There was no significant change in blood pressure of group A throughout the study period. At 4-6 week, blood pressures of group B and D increased significantly whereas blood pressure of group C was reduced as depicted in Figure-1 below.

Mean and standard deviation of serum creatinine concentration of all groups are given in Table-II and Figure-2 below.

Serum creatinine concentration of all the four groups did not change much with time. ANOVA revealed that difference among groups was not significant at any time throughout the study period. Paired t-test was applied to compare changes in serum creatinine concentration within each group with time. Serum creatinine concentration of all groups did not change significantly during any comparison time.

Mean and standard deviation of urinary volume of all groups are given in Table-III and Figure-3. Urinary volume of all the four groups did not change much with time. ANOVA revealed that difference among groups was not significant at any time throughout the study period.

Paired t-test was applied to compare changes in urinary volume within each group with time. Urinary volume of all groups did not change significantly during any comparison time.

Mean and standard deviation of urinary creatinine concentration of all groups are given in Table-IV and Figure-4. Urinary creatinine concentration of group A did not change much but that of group B increased and of group C and D decreased towards end of study. ANOVA revealed that difference among groups was not significant at any time throughout the study period (Table-IV).

Paired t-test was applied to compare changes in urinary creatinine concentration within each group with time. There was no significant change in urinary creatinine of any group throughout the study period.

Mean and standard deviation of creatinine clearance of all groups are given in Table-V and Figure-5. Creatinine clearance of group A, B and C did not change much with time but that of group D decreased towards end of study period. ANOVA revealed that difference among groups was not significant at any time throughout the study period.

		Group A	Group B	Group C	Group D	*p*-Value by ANOVA
WEEK 0	MEAN	120.41	125.48	117.7	117.98	0.167
	SD	10.97	11.20	10.84	10.86	
WEEK 4	MEAN	116.93	163.88	151.01	150.98	0.000
	SD	10.81	12.80	12.28	12.28	
WEEK 6	MEAN	118.7	162.5	121.33	168	0.000
	SD	10.89	12.74	11.01	12.96	

Table-I. Effect of *Bryophyllum pinnatum* and diclofenac on systolic blood pressure (mean±SD) of hypertensive rats (n=6)

Week	Groups								p value
	A		B		C		D		By
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA
0	0.82	0.15	0.72	0.19	0.70	0.13	0.63	0.15	0.270
4	0.80	0.09	0.72	0.19	0.60	0.13	0.60	0.14	0.067
6	0.77	0.12	0.68	0.15	0.70	0.13	0.62	0.15	0.324

Table-II. Effect of *Bryophyllum pinnatum* gel and diclofenac on serum creatinine concentration (mg/dl) of hypertensive rats (n=6)

A= normal control, B= hypertensive control, C = Bryophyllum pinnatum treated, D= diclofenac treated

Week	Groups								*p*-Value
	A		B		C		D		By
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA
0	5.77	1.05	5.72	1.80	5.40	2.69	5.63	2.37	0.990
4	5.83	0.41	5.70	0.28	5.50	0.35	5.60	0.51	0.518
6	5.87	0.60	5.77	0.23	5.40	0.94	5.17	0.59	0.234

Table-III. Effect of *Bryophyllum pinnatum* and diclofenac on urinary volume (ml) of hypertensive rats (n=6)
A= normal control, B= hypertensive control, C= Bryophyllum pinnatum treated, D= diclofenac treated

Week	Groups								*p*-Value
	A		B		C		D		By
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA
0	59.67	34.12	65.83	27.07	65.00	44.74	67.17	43.76	0.987
8	63.83	38.42	68.83	12.43	63.17	16.09	64.67	11.24	0.972
10	61.67	39.97	73.67	6.31	53.62	37.26	41.48	22.12	0.317

Table-IV. Effect of *Bryophyllum pinnatum* and diclofenac on urinary creatinine concentration (mg/dl) of hypertensive rats (n=6)
A= normal control, B= hypertensive control, C= Bryophyllum pinnatum treated, D= diclofenac treated

Week	Groups								*p*-Value
	A		B		C		D		By
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	ANOVA
0	39.98	24.28	59.52	46.10	53.77	48.19	55.13	36.31	0.850
8	47.47	29.71	57.77	17.79	60.35	21.15	62.58	15.56	0.640
10	51.98	41.59	65.95	21.06	50.37	39.07	34.53	18.72	0.420

Table-V. Effect of Bryophyllum pinnatum and diclofenac on creatinine clearance (dl/day) of hypertensive rats (n=6)
A= normal control, B= hypertensive control, C= *Bryophyllum pinnatum treated*, D= diclofenac treated

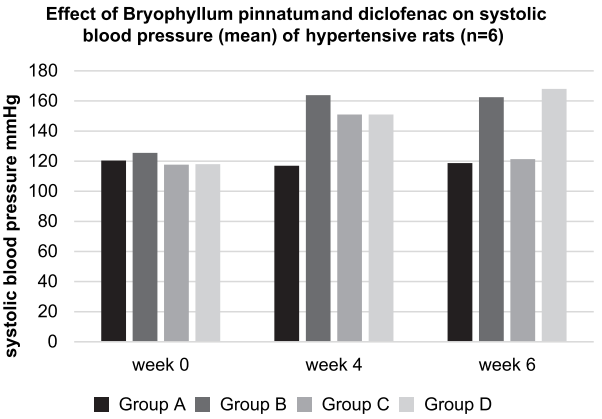

Figure-1. Effect of *Bryophyllum pinnatum* and diclofenac on systolic blood pressure (mean) of hypertensive rats (n=6) A= normal control, B= hypertensive control, C = Bryophyllum pinnatum treated, D= diclofenac treated

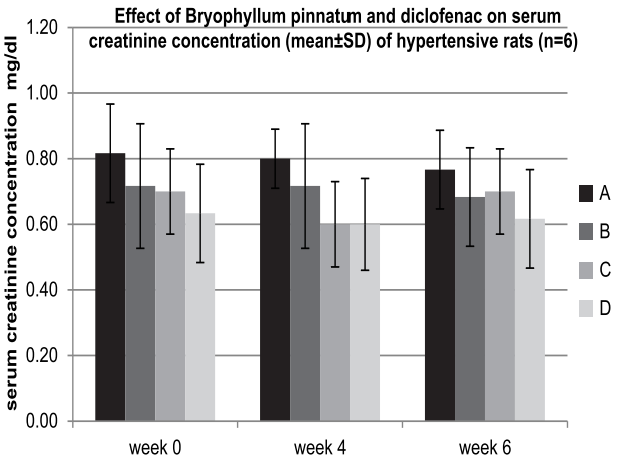

Figure-2. Effect of *Bryophyllum pinnatum* and diclofenac on serum creatinine concentration (mean±SD) of hypertensive rats (n=6)

Paired t-test was applied to compare changes in creatinine clearance within each group with time. There was no significant change in creatinine clearance of any group throughout the study period.

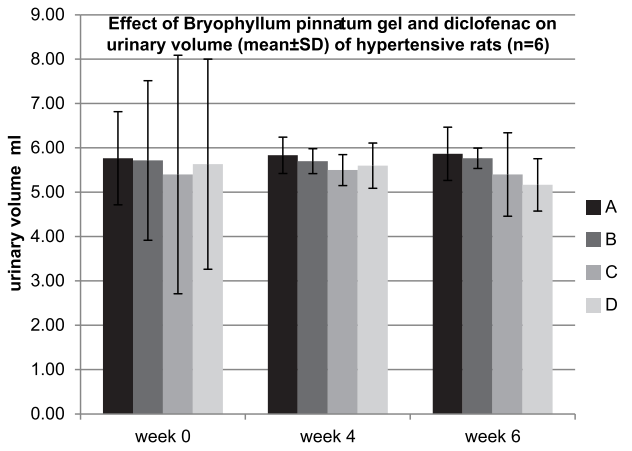

Figure-3. Effect of *Bryophyllum pinnatum* and diclofenac on urinary volume (mean±SD) of hypertensive rats (n=6)

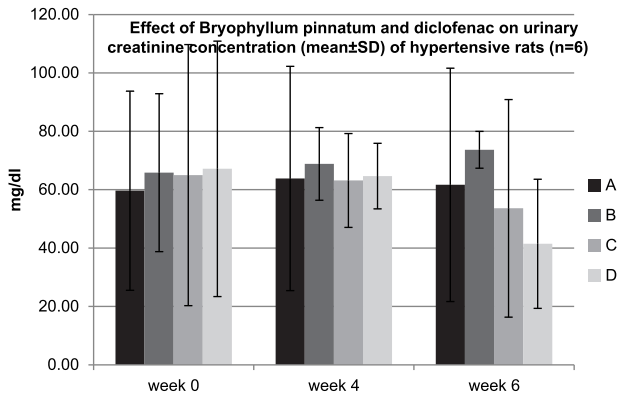

Figure-4. Effect of *Bryophyllum pinnatum* and diclofenac on urinary creatinine concentration (mean±SD) of hypertensive rats (n=6)

A= normal control, B= hypertensive control, C= Bryophyllum pinnatum treated, D= diclofenac treated.

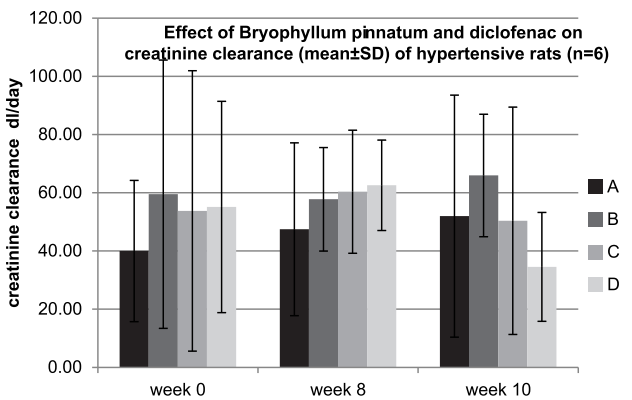

Figure-5. Effect of *Bryophyllum pinnatum* and diclofenac on creatinine clearance (mean±SD) of hypertensive rats (n=6)

A= normal control, B= hypertensive control, C= Bryophyllum pinnatum treated, D= diclofenac treated

DISCUSSION

All civilizations had used plants and herbs to treat infectious and metabolic diseases. Plants are the main pillar for the study and development of modern drugs, and medicinal plants have been recognized as the vital source to treat diseases all over the world. Hypertension is a metabolic disease of dubious etiology. The risk factors may be acquired or genetic. The identification of risk factors is significant in understanding the disease etiology. The studies have shown that certain Biochemical and Pharmacological factors affect the progression of the disease. The chronic uncontrolled Hypertension is one of the striking reasons for the development of nephropathic changes, later leads to permanent damage to the renal tissue.[14] The best parameters for the evaluation of renal function are the determination of serum creatinine and creatinine clearance.[15] Therefore, there is a dire need for the cheap, effective and safe antihypertensive agents from plants and other natural sources.

The result of the present study indicates that *Bryophyllum pinnatum* leaf aqueous extract caused significant reduction in the blood pressure in hypertensive rats. The study also indicates that the *Bryophyllum pinnatum* imparts no effect on serum creatinine levels, 24 hours urinary creatinine and creatinine clearance in hypertensive rats. This shows that it has no effect on renal function test and the extract of Bryophyllum pinnatum is safe for renal tissue.

It is a comparative study of *Bryophyllum pinnatum* with diclofenac. Paired t-test was applied to compare changes in creatinine clearance within each group with time. There was no significant change in creatinine clearance of any group throughout the study period.

CONCLUSION

In conclusion, we have reported the antihypertensive effects of *Bryophyllum pinnatum* with no effect on creatinine clearance in hypertensive rats. Although it does not show the confirmed functional effects of the *Bryophyllum pinnatum*, our results indicate that it may have a role in treatment of hypertension. Further studies

with increased number of parameters are required to verify different effects of this plant. In future, additional studies of *Bryophyllum pinnatum* on different animals may be supportive to investigate the mechanism of action of the aqueous leaf extract of the plant and its different effects in hypertensive models of animals.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Farwa Naqvi	Abstract, Literature review & methodology.	
2	Iram Imran	Perfoemd experiments & methodology.	
3	Bilal Habib	Performed experiments, Compile & analyze resutls.	
4	Summyia Sadia	Typhographical error & brief review.	
5	Zaib	Typhographical error & brief review.	
6	Sultan Sikandar	Helped in finding resutls, Helped in performing experiments.	