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Comparative Evaluation of *Aloe vera* and Diclofenac on Body Weight, Blood Pressure and Renal Function of Hypertensive Rats

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ABSTRACT

Introduction: NSAIDs are known to cause salt and water retention leading to hypertension and renal impairment. *Aloe vera* gel has been used in medicinal preparations for decades. Limited data is available regarding effect of *Aloe vera* on renal function. There is a need to search this aspect of *Aloe vera*, to use it judiciously. **Aims & Objectives:** To estimate and compare the effects of *Aloe vera* and diclofenac on systolic blood pressure and renal functions of hypertensive rats. **Place and duration of study:** This study was conducted at Post Graduate Medical Institute Lahore, Sargodha Medical College, Sargodha and Department of Pharmacy, University of Sargodha for the period of three months. **Material & Methods:** Male Sprague Dawley rats (n=24) were divided into four groups; Group A (Normal control), Group B (Hypertensive control), Group C (*Aloe vera* treated) and Group D (Diclofenac treated). Hypertension was induced in groups B, C and D by 20% sucrose diet in 8 weeks. After induction of hypertension, distilled water, dried *Aloe vera* gel 400 mg/kg and diclofenac 12 mg/kg were given orally to group B, C and D respectively for 2 weeks as a single morning dose. Body weight and systolic blood pressure were measured weekly, while serum creatinine, creatinine clearance and urinary proteins were estimated and compared at 0, 8 and 10 weeks. Data was analyzed using SPSS 23 and *p* value of ≤0.05 was considered significant. **Results:** Diclofenac decreased body weight of rats non-significantly and increased systolic blood pressure significantly ($p< 0.03$) whereas *Aloe vera* increased body weight significantly ($p<0.012$) and had no significant effect on systolic blood pressure. Diclofenac treated group showed deterioration of renal function as compared to *Aloe vera* treated group numerically. **Conclusion:** *Aloe vera* may be safer anti-inflammatory agent than diclofenac for treatment of chronic inflammatory conditions if the patient also has hypertension or renal disease.

Key words: Systolic BP, *Aloe vera*, Diclofenac, Serum creatinine, Creatinine clearance, Urinary proteins

INTRODUCTION

Hypertension is considered as one of the major causes in the development of cardiovascular diseases worldwide.[1] However, even in the presence of potent antihypertensive agents, large numbers of patients in actual clinical practice still suffer with uncontrolled hypertension. At present, it is roughly estimated that about 1.4 billion people worldwide have hypertension (>140/90 mmHg), and this number is expected to increase to 1.6 billion by 2025.[2]

The National Health Survey of Pakistan estimated that hypertension affects 18% of adults and 33% of people above 45 years old.[3] In another report, it was shown that 18% of people in Pakistan suffer from hypertension. It was also mentioned that only 50% of the people with hypertension were diagnosed and that only half of those diagnosed were ever treated. Thus, only 10 % of hypertension cases were adequately controlled.[3] Hypertension can easily be controlled by regular physical exercise, salt restriction and good compliance to antihypertensive drugs. Control of hypertension considerably decreases the risk of damage to target organs such as kidneys, heart, brain, blood vessels and eyes.[4]

One of the reasons for unsuccessful control of hypertension is long term use of NSAIDS in co-existing conditions like arthritis and gout.[5] NSAIDs are commonly used in Pakistan as analgesic, anti-inflammatory and anti-pyretic agents. They also

have many side effects such as exacerbation of asthma symptoms, increased bleeding tendency and gastric and duodenal ulceration.[6] NSAIDs are known to cause salt and water retention which eventually cause raised blood pressure and impaired renal function.[7] One of the most commonly used NSAIDs is diclofenac which like all other NSAIDs leads to hypertension and impaired renal function in human as well as animal studies.

Aloe vera gel is used as anti-inflammatory agent since long. This action is due to decreased prostaglandin E2 synthesis from arachidonic acid by inhibition of cyclooxygenase pathway.[8] This is similar to mechanism of action of NSAIDs. *Aloe vera* gel is clinically used as an anti-inflammatory agent in wound healing, gastric ulcers and ulcerative colitis,[9] but its effect on blood pressure in hypertensive subjects or animals has not been evaluated.

This study was conducted to compare the effect of *Aloe vera* gel and diclofenac on blood pressure and renal functions of hypertensive rats.

MATERIAL AND METHODS

This was an experimental study conducted at Post Graduate Medical Institute, Lahore and Sargodha Medical College, Sargodha after approval from institutional ethical committee. Duration of intervention after acclimatization was 10 weeks. Sample size was calculated by taking mean± SD of sodium excretion, diclofenac versus placebo, using formula for calculation of two independent samples at 90% power of study and 5% level of significance. Sample size calculated was six animals in each group. Male Sprague Dawley rats of 8-10-week age were included. Any rat with any significant sign of disease was excluded from study. Simple random sampling using balloting method was used for division of rats into 4 groups (A-D). Normal rat chow was purchased from Tollinton market, Lahore, and sucrose from general grocery store. Sprague Dawley rats were obtained from University of Agriculture Faisalabad. They 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 hours was maintained and animals were given free access to food and tap water.

Aloe vera plants were purchased from a nursery and identified by Department of Botany, University of Sargodha. Leaves of *Aloe vera* were washed thoroughly with water and cut to exclude the drained juice containing aloin. The edges of the leaves were cut and then split to extract the pulp. The pulp was mixed in a blender and sieved through muslin cloth. Gel was air dried. Dried gel was stored at 4°C .

Rats were divided into four experimental groups named as normal control (Group A), hypertensive control (Group B), *Aloe vera* treated (Group C) and diclofenac treated (Group D) respectively. Group A was given normal rat chow throughout the study period whereas rats in group B, C and D received a basic diet of ground regular rat chow containing sucrose (20% w/w)[10] and became hypertensive in eight weeks. Cut of value for systolic hypertension was taken as 140 mm Hg. After induction of hypertension, rats in group B, C and D received 0.5 ml distilled water, 400 mg/kg *Aloe vera* gel powder[11] and 12 mg/kg diclofenac powder[12] respectively orally as a single morning dose for two weeks.

Body weight of animals was recorded at baseline and then weekly throughout the study by Shimadzu ELB 3000 digital weighing balance. Blood pressure of animals was recorded at baseline and then weekly throughout the study. Systolic blood pressure was measured by tail cuff[13] using noninvasive blood pressure controller (ML125R) attached to computer based data recording system. Five Systolic blood pressure readings were measured by tail cuff and mean systolic blood pressure was calculated for each rat. At 0, 8 and 10 weeks animals were kept in individual cages for collection of 24 hours urine, after which they were anesthetized with chloroform and two ml blood was drawn through cardiac puncture. Serum and urinary creatinine levels were estimated by kinetic Jaffé method.[14] Twenty-four hour urine was collected at 0, 8 and 10 weeks by keeping animals in individual cages for calculation of creatinine clearance which was calculated using following formula:

$$\text{Clearance} = \frac{\text{urine concentration x urine flow rate}}{\text{plasma concentration}}$$

Urine was tested for proteins by dip stick method. The result is read in plus system as negative trace, and 1+ to 4+.

Statistical analysis:

Data was analyzed using SPSS 23. Normality was tested by Shapiro-Wilk normality test and data presented as mean ± SD. Normally distributed data was compared using ANOVA. Post hoc Tukey's test was applied for comparison among groups. Changes in parameters at different times in each group were compared by t-test. Kruskal Wallis ANOVA, a non-parametric method, was applied to data of urinary proteins. A p value of ≤ 0.05 was considered significant.

RESULTS

The weight of rats was not similar at day 0 after random allocation so it was converted into percentage and was considered as 100%. Mean and standard deviation of weights after conversion into percentages are given in figure 1. Body weight increased non-significantly in group A from 0-10 week whereas increase was significant in group B, C and D from 0-8 week. From 8-10 week, increase in body weight was significant only in group C.

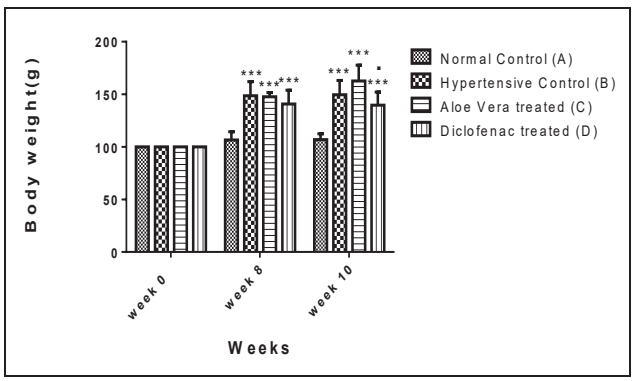

Fig-1: Effect of *Aloe vera* gel and diclofenac on body weight (mean±SD) of hypertensive rats (n=6)

*** *p* value <0.001 Versus Group A

● *p* value <0.05 Versus Group C

Blood Pressure:

Mean and standard deviation of blood pressure are given in Fig-2. There was no significant change in blood pressure of group A throughout the study period. Blood pressures of group B, C and D increased significantly from 0-8 and 0-10 weeks. Blood pressures of group B, C and D increased from 8-10 week but this increase was significant in group D only and non-significant in group B and C.

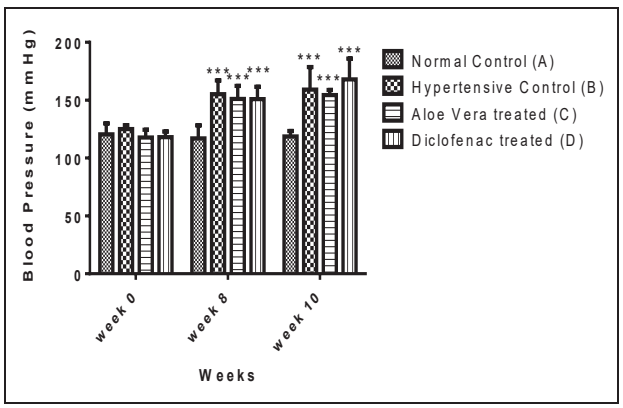

Fig-2: Effect of *Aloe vera* gel and diclofenac on systolic blood pressure (mean±SD) of hypertensive rats (n=6)

*** *p* value <0.001 Versus Group A

Serum Creatinine

Serum creatinine concentration of all groups did not change significantly during any comparison time as shown in Fig-3.

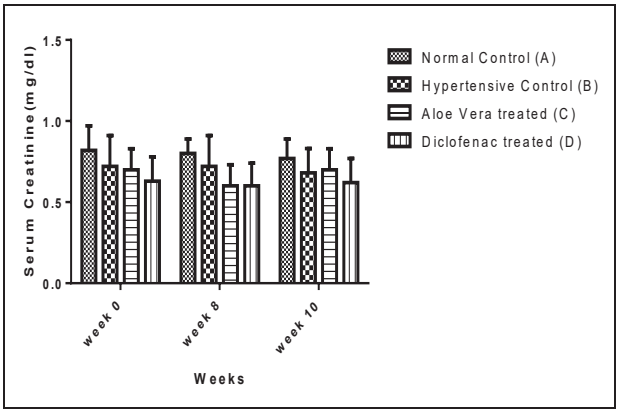

Fig-3: Effect of *Aloe vera* gel and diclofenac on serum creatinine (mean±SD) of hypertensive rats (n=6)

Creatinine Clearance

There was no significant change in creatinine clearance of any group throughout the study period as shown in Fig-4.

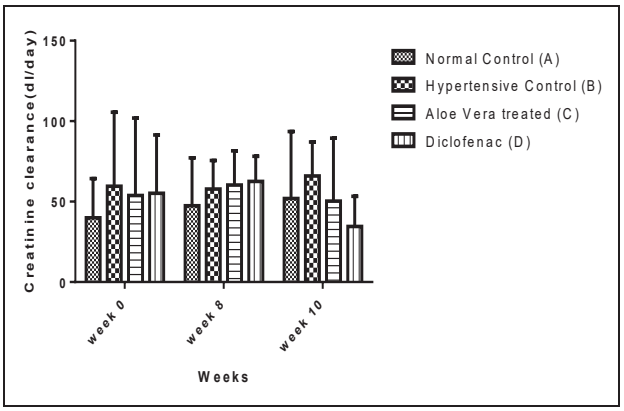

Fig-4: Effect of *Aloe vera* gel and diclofenac on creatinine clearance (mean±SD) of hypertensive rats (n=6)

Urinary Proteins

Grade wise number and percentages of urinary proteins of all groups are given in Fig-5.

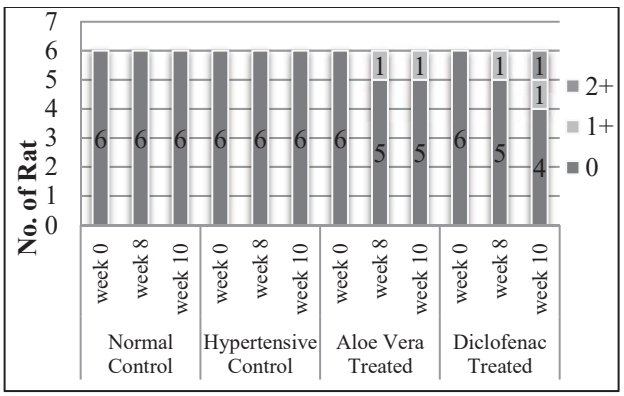

Fig-5: Effect of *Aloe vera* gel and diclofenac on urinary proteins of hypertensive rats (n=6)

DISCUSSION

NSAIDs are used in several diseases due to their anti-inflammatory action but have known adverse effect of increasing blood pressure[15] and disturbing renal functions.[7] *Aloe vera* is a herb with known anti-inflammatory action.[16] This study was conducted to compare the effects of *Aloe vera* and diclofenac on blood pressure and renal functions of hypertensive rats. Hypertension was induced by high sucrose diet using standard methodology. [17] After induction of hypertension in eight weeks, *Aloe vera* gel and diclofenac were administered for two weeks. It was deduced from this study that after treatment of hypertensive rats with *Aloe vera* gel body weight increased and blood pressure did not deteriorate, while with diclofenac body weight decreased and blood pressure increased. Effect on renal function was only numerically worse with diclofenac.

Body weight of rats increased during 0-8 week period with p value <0.001 in all groups receiving high sucrose diet. During 8-10 weeks period, there was significant increase in the body weight of *Aloe vera* treated rats with p value of 0.0120 which is similar to a study which shows significant increase in body weight of *Aloe vera* treated rats with p value <0.001.[18] Body weight of diclofenac treated rats decreased during 8-10 weeks whereas body weight of rats in normal control and hypertensive control groups increased. These results are supported by another study in which diclofenac halted the weight gain in rats[19] which may be due to decreased appetite as a result of gastrointestinal injury. At week 10 body weight of rats in diclofenac treated group was significantly lowered as compared to *Aloe vera* treated group with p value <0.05 but there was no significant difference in body weight of rats in hypertensive control group when compared with body weight of both *Aloe vera* and diclofenac treated groups.

In present study, there was no significant change in blood pressure of normal control group throughout the study period, whereas blood pressure in all groups receiving sucrose diet was significantly increased with p value of <0.001 after eight weeks of sucrose diet confirming that hypertensive model is successfully developed. In present study, mean increase of systolic BP from 8-10 weeks was 4 mmHg in hypertensive control, 3 mmHg in *Aloe vera* treated group whereas in diclofenac treated group, mean blood pressure increased 17mmHg with p value of 0.03. In accordance with this study, intra-peritoneal injection of diclofenac has shown a significant (p <0.001) rise in blood pressure of hypertensive rats.[20] In another study, diclofenac has shown increase in blood pressure of elderly human volunteers with a p value of 0.08.[21] These results are contrary to a study in which diclofenac did not change blood pressure of hypertensive rats[22] which may be because of a very small dose (1mg/kg) of diclofenac given for fifteen days.

In present study *Aloe vera* had no significant effect on BP. Supporting this result there is a study in which *Aloe vera* given for 12 weeks did not affect blood pressure of type-2 diabetics[23] and another study in which single dose of *Aloe vera* did not affect blood pressure of healthy volunteers.[24] There is no data for effect of *Aloe vera* on BP of hypertensive subjects.

Serum creatinine level increased non-significantly in diclofenac group whereas it decreased non-significantly in both the control groups during 8-10 week period. However in another study, diclofenac increased serum creatinine significantly in rats after 7 days of treatment[25] which can be due to much higher doses of diclofenac used as compared to present study. Serum creatinine of *Aloe vera* group also increased non-significantly which is in accordance with a study done on type 2 diabetics.[23]

Creatinine clearance was calculated by the formula which is the default method of assessing renal function. The results of this study showed that both *Aloe vera* and diclofenac non-significantly decreased creatinine clearance but the effect of diclofenac was numerically more (28.05) as compared to the effect of *Aloe vera* (9.98). Similar to this study, a study on healthy volunteers has shown no significant change in creatinine clearance with *Aloe vera.*[26] Another study has shown similar non-significant decrease in creatinine clearance in rats after diclofenac treatment.[20] Had the study been conducted for longer duration, results of serum creatinine and creatinine clearance may have become significant.

There was no significant change in concentration of urinary proteins in all the groups throughout the study period. However, in *Aloe vera* treated group the concentration of urinary proteins remained constant from 8-10 weeks period whereas in diclofenac treated group, concentration of urinary proteins increased in this period. This shows tendency of diclofenac to produce damaging effect on renal parenchyma as shown in another study.[27]

These results have shown that there was significant worsening of hypertension with diclofenac whereas *Aloe vera* gel did not elevate the blood pressure of hypertensive rats more than that in hypertensive control group.

Mechanism of action of diclofenac is inhibition of prostaglandin synthesis resulting in decreased level of renal prostaglandins leading to impaired renal functions and elevation of blood pressure. *Aloe vera* is also an anti-inflammatory agent and one of its mechanism of action is inhibition of prostaglandins as well which may lead to increased blood pressure, but this has not been demonstrated in present study. In this study, crude form of *Aloe vera* was used which has more than 75 active ingredients including acemannan and sterols[28] which may have counteracted the effects of prostaglandin in *Aloe vera* on renal functions.

Limitations of the study are that it was for shorter duration and only single dose of *Aloe vera* gel was administered.

CONCLUSION

Results of this study indicate that *Aloe vera* gel did not deteriorate blood pressure of hypertensive rats, while diclofenac did. Renal function worsened non-significantly by both treatments, but studies of longer duration are required for validation.

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