

## RESEARCH ARTICLE

# Evaluation of diuretic, saluretic and natriuretic activity of hydrochlorothiazide in combination with misoprostol in Wistar rats

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### ABSTRACT

**Background:** Renal vasodilatation by prostaglandins is well proven facts. Furosemide is better diuretics due to their additional effect on intrarenal hemodynamic changes brought about by increase in local prostaglandin synthesis. No such changes occur with thiazide group of diuretics. Hence, we want to analyze the diuretic effect of hydrochlorothiazide in combination with prostaglandin analog misoprostol. **Aims and Objectives:** This study aims to evaluate the diuretic, saluretic, and natriuretic activity of hydrochlorothiazide in combination with misoprostol. **Materials and Methods:** Four groups of Wistar rats with six animals in each group were grouped. One group served as the control and the other three groups received furosemide, hydrochlorothiazide, and hydrochlorothiazide with misoprostol, respectively. Urine output, urinary electrolytes, saluretic activity, and natriuretic of all groups were estimated and then compared with the standard and control groups. **Results:** There was a statistically difference in the urinary output between groups as determined by one-way ANOVA ( $F = 33.413, P \leq 0.001$  and  $F = 260.267, P = 0.001$ ) at 5 and 24 h, respectively. The Lipschitz value showed that standard group and test Group 4 indices were  $>2.0$  showing potent diuretic action. There was a statistically significant difference in the urinary excretion of all the electrolytes between groups as determined by one-way ANOVA ( $F = 645.087, P \leq 0.001; F = 360.49, P \leq 0.001; F = 366.974, P \leq 0.001$ ) for the levels of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ , respectively. It has been found that the saluretic activity was significant in all three groups when compared with control group. However, they do not show significant changes in natriuretic activity. **Conclusion:** This study has shown that the diuretic activity and diuretic index of hydrochlorothiazide can be increased when it is given in combination with prostaglandin analog misoprostol and also found to be almost equivalent to standard drug furosemide effect. There was also a significant effect in the excretion of electrolytes in urine and significant saluretic activity that favors their use in edematous conditions. Saluretic activity of hydrochlorothiazide with misoprostol was also similar to furosemide. Since both the drugs are already approved and safely used for many decades, we can further do a clinical trial to confirm these findings and can be used as a combination of old drugs for a new indication.

**KEY WORDS:** Diuretic Index; Misoprostol; Hydrochlorothiazide; Furosemide

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### INTRODUCTION

Diuretics are among the widely used drugs which cause a net loss of sodium and water in urine.<sup>[1]</sup> The increased water loss is actually secondary to the increased excretion of sodium chloride.<sup>[2]</sup> They have a central role in the treatment of edema related to congestive cardiac failure, renal failure,

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cirrhosis of liver, etc. Lots of plant extracts have been evaluated for their diuretic effect, but none succeeded as loop diuretics like furosemide. Many research works have proven that plant extracts showed significant diuretic activity, and they attributed it due to the involvement of prostaglandins.<sup>[3-5]</sup> Renal vasodilatation by prostaglandins is well proven facts. Furosemide, in addition to their prominent tubular transport inhibitory action, also causes important intrarenal hemodynamic changes by inducing local prostaglandin synthesis. Hence, they transiently increase renal blood flow and also cause an increase in venous capacitance which provides quick relief in pulmonary edema. This effect is masked by the concomitant use of nonsteroidal anti-inflammatory drugs.<sup>[1,6]</sup> This type of intrarenal hemodynamic changes does not occur with thiazide group of diuretics. Instead of searching for a new molecule, we thought that of searching old drugs with equal or more effects as the standard drug furosemide. Hence, the need for this study was to analyze whether the addition of prostaglandin analogs to the thiazides will improve its diuretic activity.

## MATERIALS AND METHODS

After obtaining permission from the institutional animal ethical committee, the experiment was carried out in the Central Animal House of our institute (CPCSEA Registration Number - 686/02/a/CPCSEA). Adult male Wistar rats with weights ranging from 200 to 250 g and 8–10 weeks of age were used for the experiment. The animals were housed in polypropylene cages (six animals per cage) under standard environmental conditions (25°C ± 1°C, 55% ± 5% humidity, and 12 h/12 h light/dark cycle). The animals were allowed free access to tap water and pellet diet.

Animals were randomly assigned into four groups each consisting of six male rats for diuretic test.

Group 1 - Control group

Group 2 - Standard drug, furosemide 25 mg/kg (Positive controls)

Group 3 - Hydrochlorothiazide 25 mg/kg

Group 4 - Hydrochlorothiazide 25 mg/kg + misoprostol 200 µg/kg

### Evaluation of Diuretic Activity

Diuretic activity was determined by Lipschitz method<sup>[7]</sup> with slight modification.

Animals were fed with standard diet and water *ad libitum*. 15 h before the experiment, food and water were withdrawn. Each group was then treated with their respective drugs. Immediately, after administration, animals were placed in metabolic cages, and their urine output was recorded after 5 and after 24 h for each group. The urinary electrolytes such as Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> were determined.

Urine volume excreted per 100 g body weight is calculated for each group. Results were expressed as the “Lipschitz-value,” i.e., the ratio T/U, in which T - the response of the test compound and U - response of the control group. Indices of 1.0 and more are regarded as a positive effect. Lipschitz values of 2.0 and more indicates more potent diuretic effect.

### Evaluation of Saluretic Activity<sup>[8]</sup>

The sum of Na<sup>+</sup> and Cl<sup>-</sup> excretion was calculated for saluretic activity. The ratio of Na<sup>+</sup>/K<sup>+</sup> was calculated for natriuretic activity. Values >2.0 indicate a favorable natriuretic effect. Ratios >10.0 indicate a potassium-sparing effect.

## RESULTS

### Diuretic Activity

The total urine output after 5 h and 24 h of each group and the diuretic index are mentioned in Table 1.

There was a statistically difference in the urinary output between groups as determined by one-way ANOVA ( $F = 33.413$ ,  $P \leq 0.001$  and  $F = 260.267$ ,  $P = 0.001$ ) at 5 and 24 h, respectively. *Post hoc* test using Bonferroni revealed that all the three groups have shown significant changes in the total urinary output when compared with the control group at 24 h. However, at 5 h, only the standard group and the Group-4 have shown a significant increase in the total urine output when compared with the control group.

The Lipschitz value was calculated, and it was found that Group 2 and Group 4 indices were >2.0 showing potent diuretic action.

### Effect on urinary electrolyte excretion

The urinary electrolytes excretion (Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>) was calculated at the end of 24 h represented in Table 2.

There was a statistically significant difference in the urinary excretion of all the electrolytes between groups as determined by one-way ANOVA ( $F = 645.087$ ,  $P \leq 0.001$ ;  $F = 360.49$ ,  $P \leq 0.001$ ; and  $F = 366.974$ ,  $P = <0.001$ ) for the levels of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>, respectively. *Post hoc* test using Bonferroni revealed that all the three groups have shown significant changes in the urinary electrolytes level when compared with the control group at 24 h.

### Effect on Saluretic and Natriuretic Activity

The saluretic activity and the natriuretic activity were calculated with the standard formula and presented in Table 3. It has been found that the saluretic activity was significant in all three groups when compared with the control group as determined by one-way ANOVA followed

**Table 1: Diuretic activity**

Duration	Group	Urinary output (ml) Mean (SD)	Diuretic index
5 h	Control	1.08 (0.12)	-
	Standard	1.83 (0.26)*	1.69
	Hydrochlorothiazide	1.38 (0.14)	1.27
	Hydrochlorothiazide+misoprostol	2.17 (0.26)*	2.00
24 h	Control	3.71 (0.29)	-
	Standard	8.21 (0.37)*	2.21
	Hydrochlorothiazide	4.46 (0.40)*	1.20
	Hydrochlorothiazide+misoprostol	8.00 (0.35)*	2.15

\*Significant  $P$  value of  $<0.05$  when compared to control group. SD: Standard deviation

**Table 2: Effect on urinary electrolyte excretion**

Urinary electrolytes	Group	Excretion (mEq/L) Mean (SD)
Na <sup>+</sup>	Control	82.17 (3.31)
	Standard	179.33 (4.08)*
	Hydrochlorothiazide	172.67 (3.20)*
	Hydrochlorothiazide +Misoprostol	177.83 (6.74)*
K <sup>+</sup>	Control	56.00 (2.53)*
	Standard	119.67 (5.65)*
	Hydrochlorothiazide	115.00 (3.63)*
	Hydrochlorothiazide +Misoprostol	117.50 (3.39)
Cl <sup>-</sup>	Control	80.67 (3.08)
	Standard	146.33 (4.50)*
	Hydrochlorothiazide	108.33 (4.84)*
	Hydrochlorothiazide +Misoprostol	140.17 (2.71)*

\*Significant  $P < 0.05$  when compared to control group. SD: Standard deviation

by Bonferroni *post hoc* test. However, they do not show significant changes in natriuretic activity in comparison with the control group.

## DISCUSSION

There was a statistically difference in the urinary output between groups as determined by one-way ANOVA ( $F = 33.413$ ,  $P \leq 0.001$  and  $F = 260.267$ ,  $P = 0.001$ ) at 5 and 24 h, respectively. The Lipschitz value showed that standard group and test Group 4 indices were  $>2.0$  showing potent diuretic action. There was a statistically significant difference in the urinary excretion of all the electrolytes between groups as determined by one-way ANOVA ( $F = 645.087$ ,  $P \leq 0.001$ ;  $F = 360.49$ ,  $P \leq 0.001$ ; and  $F = 366.974$ ,  $P \leq 0.001$ ) for the levels of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>, respectively. It has been found that the saluretic activity was significant in all three groups when compared with control group. However, they do not show significant changes in natriuretic activity.

At 5 h duration, the standard group receiving furosemide and the test Group 4 receiving hydrochlorothiazide with misoprostol have shown a significant increase in the total urine output when compared with the control group. However, at 24 h, all the groups showed a significant rise in urinary volume when compared with the control. *Post hoc* test using Bonferroni also revealed that no significant differences between standard group and Group 4 at 24 h. This shows that they have similar effects in the urinary volume and shows similar diuretic activity. Furthermore, when the Lipschitz value was calculated, it was found that Group 2 and Group 4 indices were almost similar and  $>2.0$  proving that the combination of hydrochlorothiazide and misoprostol has similar potent diuretic action as the standard drug furosemide. The hydrochlorothiazide group showed only 1.27 as diuretic index, but this was controversial to another study conducted by Gadge *et al.*<sup>[9]</sup> with plant extract and in that they have shown hydrochlorothiazide diuretic index was more than 2 at 5 and 24 h.

Excretion of electrolytes is very important like the amount of water excretion in urine, particularly when it is used in the treatment of edema related to congestive cardiac failure. However, the loss of potassium should be avoided. Hence, when the estimated urinary electrolytes were analyzed statistically, we noted that there was a statistically significant difference in the urinary excretion of all the electrolytes between groups when compared with the control group. *Post hoc* analysis also proved the same in comparison with the control group. It also revealed that no significant differences between standard group and Group 4 which suggest that they have similar effects in the excretion of the urinary electrolytes, and potassium loss is also similar to that of the standard drug.

It has been found that the saluretic activity was significant in all three groups when compared with the control group as determined by one-way ANOVA followed by Bonferroni *post hoc* test.

Values of natriuretic index more than 2.0 indicate favorable natriuretic activity and more than 10.0 means potassium-sparing effect. Standard and test groups had a natriuretic

**Table 3: Effect on saluretic and natriuretic activity**

Activity	Group	Mean (SD)	F ratio	P value
Saluretic activity (Na <sup>+</sup> + Cl <sup>-</sup> )	Control	162.83 (3.76)	1036	<0.001
	Standard	325.67 (6.83)*		
	Hydrochlorothiazide	281.00 (4.65)*		
	Hydrochlorothiazide+Misoprostol	318.00 (6.99)*		
Natriuretic activity (Na <sup>+</sup> /K <sup>+</sup> )	Control	1.47 (0.06)	0.433	0.732
	Standard	1.50 (0.08)		
	Hydrochlorothiazide	1.50 (0.07)		
	Hydrochlorothiazide+Misoprostol	1.51 (0.08)		

\*Significant *P* value of <0.05 when compared to control group. SD: Standard deviation

index of 1.5 and did not show any significant changes in natriuretic activity in comparison with the control group. This has proven that they do not possess significant natriuretic as well as potassium-sparing effect.

### Strengths

Instead of the search of new drug molecule or any plant extract, we have obtained increased diuretic effect with a combination of two old existing drugs. The safety profile of them is also well known. Hence, this can be used safely in humans after a confirmatory trial.

### Limitations

We cannot extrapolate the findings found in animals to humans. It is also not necessary that this combination will show similar effect in humans as in rats.

### CONCLUSION

This research work has shown that the diuretic activity and diuretic index of hydrochlorothiazide can be increased when it is given in combination with prostaglandin analog misoprostol and also found to be almost equivalent to standard drug furosemide effect. There was also significant effect in the excretion of electrolytes in urine that favors their use in edematous conditions associated with congestive cardiac failure and renal failure. Saluretic activity of hydrochlorothiazide with misoprostol was also similar to furosemide. Since both the drugs are already approved and safely used for many years, we can do a clinical trial to confirm these findings and can be used as a combination of old drugs for a new indication.

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