

## EVALUATION OF DIURETIC ACTIVITY OF HYDROCHLOROTHIAZIDE - $\gamma$ -CYCLODEXTRIN INCLUSION COMPLEX IN WISTAR RATS

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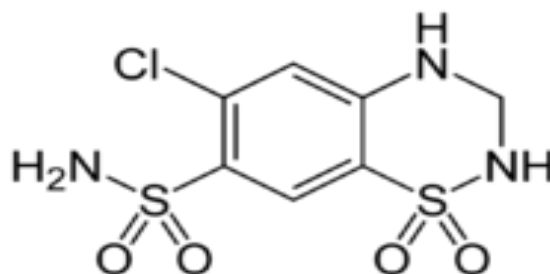
### ABSTRACT

The thiazide diuretics are the first line treatment recommended for hypertension, in particular hydrochlorothiazide (HCT). The aim of the present study is to evaluate the diuretic activity of HCT-  $\gamma$ -Cyclodextrin (HCT- $\gamma$ CD) inclusion complex in comparison with HCT alone. Vehicle (distilled water), HCT-  $\gamma$ CD complex (10mg/kg) and HCT alone (10mg/kg) were administered orally to male wistar rats and their urine output was quantitated after 24 hours. Volume, pH, conductivity, and Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup> concentrations of urine were measured. The results showed significant improvement of the urine output of HCT- $\gamma$ CD complex compared to HCT alone respectively  $5.52 \pm 0.52$  versus  $4.3 \pm 0.28$  ml/100g/24h . Urinary electrolyte excretion and conductivity was also increased. Significant diuretic ( $p < 0.05$ ) and electrolyte excretion effects ( $p < 0.05$ ) were observed in treated groups by HCT- $\gamma$ CD complex compared to the control and reference standard (hydrochlorothiazide). Therefore, we can conclude that HCT- $\gamma$ CD complex produced notable diuretic effect.

**KEY WORDS:** Hydrochlorothiazide,  $\gamma$ - cyclodextrin, diuretic activity.

## INTRODUCTION

The absolute risk of cardiovascular events is mainly determined by high blood pressure. Antihypertensive therapy enable to reduce considerably the risk of developing cardiovascular that cause a high mortality rate in the industrialized countries <sup>[1]</sup>, Pharmacological treatment of hypertension consists in the use of drug therapies including diuretics, beta-blockers, calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor (AT1) antagonist (ARA) <sup>[2-4]</sup>. Hydrochlorothiazide (HCT) is a diuretic and antihypertensive within the class of benzothiadiazine drug that reduces plasma volume by increasing the excretion of sodium, chloride and water and, to a lesser extent, that of potassium as well <sup>[5,7]</sup>. Fig.1 shows the structural formulae of hydrochlorothiazide.



**Fig. 1. Molecular structure of Hydrochlorothiazide**

Cyclodextrins (CDs) are a cyclic oligosaccharides, consisting of six  $\alpha$ -cyclodextrin, seven  $\beta$ -cyclodextrin, eight  $\gamma$ -cyclodextrin or more glucopyranose units linked by  $\alpha$ -(1,4) bonds <sup>[8-10]</sup>. CDs are able to form inclusion complexes with poorly water-soluble drugs. These inclusion complexes have been shown to improve stability, solubility and bioavailability <sup>[11,12]</sup>. In a previously conducted study, we tested the effect of  $\gamma$ -cyclodextrin ( $\gamma$ CD) on the aqueous solubility, dissolution rate, bioavailability and intestinal permeability using the Ussing chamber technique of the hydrochlorothiazide <sup>[13]</sup>. The results demonstrated that the aqueous solubility, bioavailability and permeability of HCT were improved significantly by its inclusion with  $\gamma$ CD <sup>[13]</sup>. The aim of this work is to compare the diuretic activity of HCT- $\gamma$ cyclodextrin complex to HCT alone.

## MATERIAL AND METHODS

### Experimental animals

The experiments were conducted on male Wistar rats with a corporal weight between 170 and 300g. During the experiments, the animals were fed ad libitum with standard food and water except when fasting was required in the course of the study. They were kept in a temperature

and humidity controlled environment ( $23 \pm 2^{\circ}\text{C}$  and  $70 \pm 5\%$ ) with a 12 h light - dark cycle in proper ventilation. The animals were acquired from the animal laboratory of Medicine and Pharmacy Faculty, Mohammed V Souissi University, Rabat. The care of the rats was in compliance with the guidelines of the guide for the care and use of laboratory animals (Commission on life science, National Research Council 1996). All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

### Diuretic test

The male wistar rats were divided into three groups: control pretreated with distilled water ( $n = 6$ ). HCT alone (reference standard) ( $n=6$ ) and HCT- $\gamma$ CD complex ( $n=6$ ) were administered by oral route. The animals were fasted overnight, with free access to drinking water only. Three groups were pre-treated with physiological saline (0.9% NaCl) at an oral dose of 5 ml/100 g body weight (BW), to impose a uniform water and salt load <sup>[14]</sup>. Suspension of HCT and HCT- $\gamma$ CD were prepared in distilled water and administrated to animals by gavage performing doses of  $10\text{mg.kg}^{-1}$  body weight. Distilled water was used as control. Immediately after administration, the rats were placed individually in the metabolic cages. Urine was collected in a graduated cylinder. The volume of urine excreted after 24h for each animal in the group is expressed as mEq/100g BW.

### Determination of electrolyte levels

$\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$  concentrations of the urine samples collected during 24h were measured using a automated chemistry analyzer (Architect c8000, abbott, Germany). The instrument was calibrated with standard solutions. Concentration of electrolytes was expressed in mEq/100g BW/24h.

### Determination of urine pH and conductivity

pH of the fresh urine samples from all the three groups was measured with a calibrated pH meter (AD1030, Romania). The conductivity was directly determined on fresh urine samples using a conductometer (Cond 340i/SET, Germany) and expressed as mS/cm/24h

### Statistical analysis

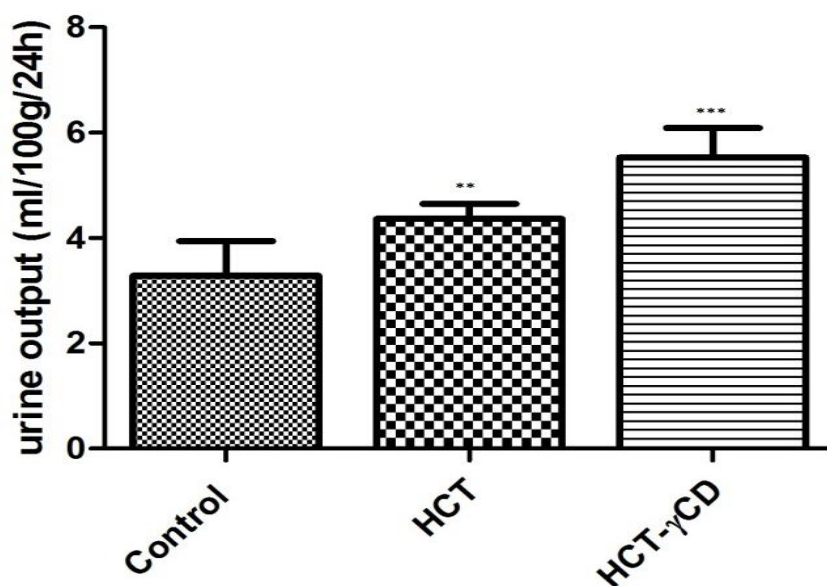
Data are expressed as mean $\pm$ S.D. (standard deviation). Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Bonferroni test, while Dannel's multiple comparison test is used to assess the comparison between control and the other groups. Significant differences were set at p values less than 0.05.

## RESULTS

The HTC- $\gamma$ CD inclusion complex was prepared by co-precipitation method in 1: 1 molar ratio. The Characterization of the inclusion complex has been demonstrated by X-ray diffraction technique and nuclear magnetic resonance <sup>[13]</sup>. In a previous study, the bioavailability of HCT alone and HCT- $\gamma$ CD inclusion complex was described, our results showed that HCT- $\gamma$ CD has higher AUC and  $C_{max}$  values than HCT alone. On the other hand, the intestinal permeability study demonstrated that a permeability of HCT was improved significantly by its inclusion with  $\gamma$ CD <sup>[13]</sup>. In this report, the effect of  $\gamma$ -Cyclodextrin ( $\gamma$ CD) on the pharmacological activity of the hydrochlorothiazide was investigated.

### Urine output, conductivity and pH

Results on the cumulative volumes of excreted urine after oral administration of the compounds are shown on Figure 2. The results showed that HCT- $\gamma$ -CD complex (10mg/100gBW) produced a notable and highly significant increase in urinary excretion of 41% ( $p < 0.05$ ) versus 24% ( $p < 0.05$ ) of reference standard HCT (10mg/100gBW) compared to control group. Thus the diuretic effect of both products are indicated by increase in both water excretion. Table 1 shows other parameters related to excretion such as the conductivity and pH. The specific conductivity, which is an indirect measure of the ionic content of the urine. Data demonstrated that HCT- $\gamma$ CD inclusion complex has a high conductivity which statistically significant ( $P < 0.05$ ) when compared to control group and thiazide group. HCT- $\gamma$ CD complex showed slight decrease in urinary pH, but was not statistically significant.



**Fig.2.** Urine output of Rats treated with water (control), HCT, HCT- $\gamma$ -CD complex.

\*\* $P < 0.01$ ; \*\*\* $P < 0.001$  compared with controls using Dannel's multiple comparison test

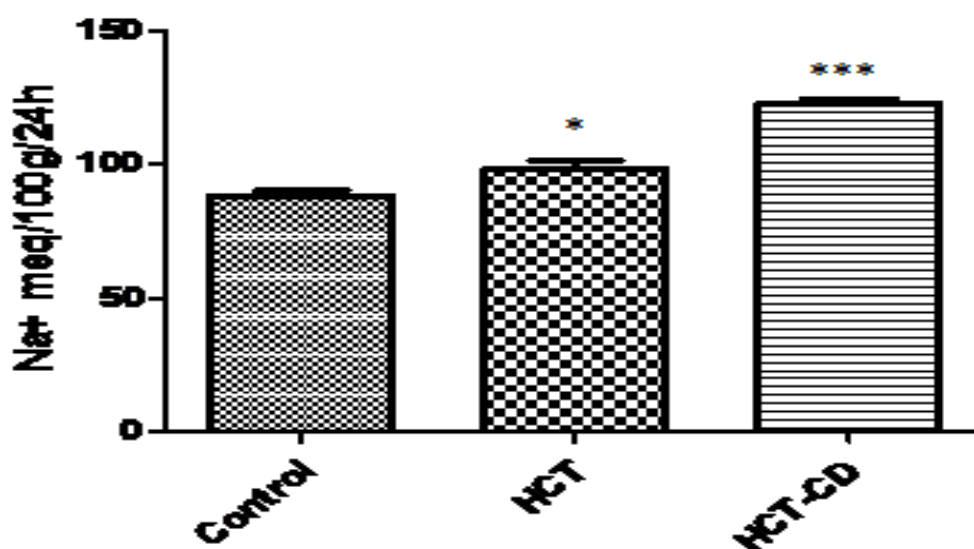
**Table 1: pH and conductivity in rats over a 24h period following oral administration of the HCT- $\gamma$ CD, HCT and control groups.**

Treatment	pH	Conductivity (mS/cm/24h)
Control	$7 \pm 0.4$	$39.3 \pm 3$
HCT 10 mg. Kg <sup>-1</sup> BW	$7.6 \pm 0.2$	$38.1 \pm 2.8^{**}$
$\gamma$ CD-HCT complex 10 mg. Kg <sup>-1</sup> BW	$8.4 \pm 0.5$	$45 \pm 4.5^{***}$

Values are expressed as mean  $\pm$  S.D., n=6. <sup>\*\*</sup>P<0.01; <sup>\*\*\*</sup>P<0.001 compared with the control group using Danneet's multiple comparison test

### Electrolyte excretion

Quantification of sodium and chloride ions in urine is one of the best methods to determine the diuretic effect of drugs [14, 15]. Figure 3 and 4 shows cumulative sodium and chloride values in excreted urine respectively. The HCT- $\gamma$ CD complex has statistically significant high Na<sup>+</sup> excretion (p< 0.05) and high Cl<sup>-</sup> (p<0.05) excretion as compared to control group. It also showed statically increase in Na<sup>+</sup> (p<0.05) and Cl<sup>-</sup> (p< 0.05) excretion compared to HCT group, which demonstrate its high efficacy than HCT alone. Figure 5 shows cumulative potassium excretion. Potassium excretion values in HCT group were less than the inclusion complex and the control but were not statistically significant (p > 0.05). Contrarily the HCT- $\gamma$ CD compound displayed a pronounced kaliuretic activity. The excretion of potassium in the urine was increased significantly (p < 0.05) as compared to control group and HCT group (p<0.05).



**Fig.3. Sodium excretion after 24h of urine collection**

\*P<0.05; <sup>\*\*\*</sup>P<0.001 compared with controls using Danneet's multiple comparison test

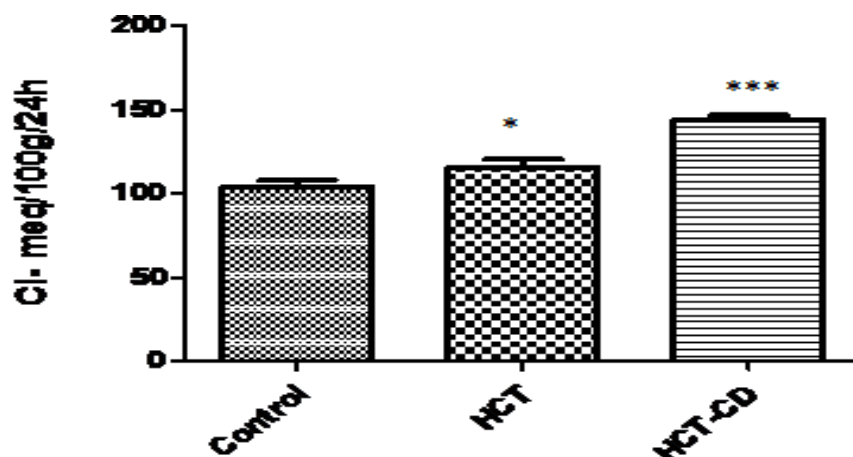


Fig.4. Chloride excretion after 24h of urine collection.

\*P<0.05; \*\*\*P<0.001 compared with controls using Dannel's multiple comparison test

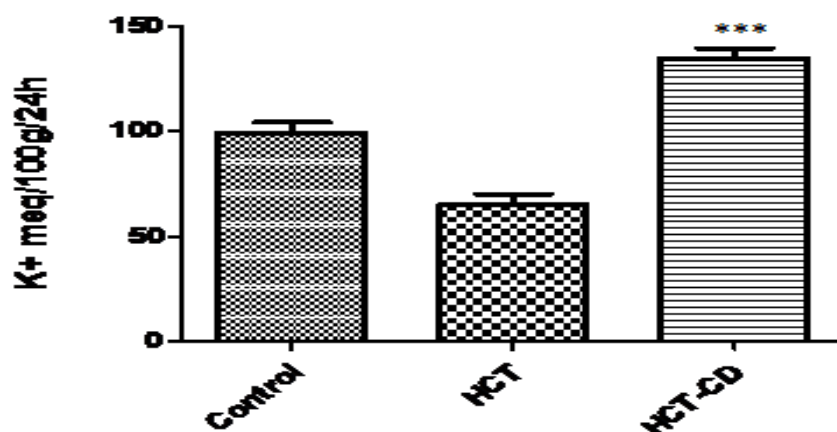


Fig.3. Potassium excretion in 24h of urine collection

\*\*\*P<0.001 compared with controls using Dannel's multiple comparison test

### Statistical analysis

The results of statistical analysis were showed in Table 2 .

Table 2: Statistical treatment of data set

Statistical tests	Variables		p-value				
			Na <sup>+</sup>	Cl <sup>-</sup>	K <sup>+</sup>	urine output	conductivity
Bonferroni	control	HCT	0.0209	0.068	0.4622	-	-
	control	HCT-γCD	1.532E-05	1.145 E-05	0.0259	-	-
	HCT	control	0.0209	0.068	0.4622	-	-
	HCT	HCT- γCD	0.0052	0.0168	0.0019	-	-
	HCT- γCD	control	1.532E-05	1.145 E-05	0.0259	-	-
	HCT-γCD	HCT	0.0052	0.0168	0.0019	-	-
Dunnet test	HCT	control	0.0131	0.0419	0.2634	0.002	0.002
	HCT- γCD	control	1.0041E-05	7.451E-05	0.0162	0	1E-05

## DISCUSSION

In the present study, the diuretic effect of HCT- $\gamma$  CD inclusion complex compared to HCT alone was evaluated in normal rats after one dose. The effect on electrolytes excretion in urine and other parameters was also evaluated. HCT- $\gamma$ CD inclusion complex produced better diuresis effect compared to HCT alone, This effect may be produced by stimulation of regional blood flow or initial vasodilation <sup>[16]</sup>, or by producing inhibition of tubular reabsorption of water and anions <sup>[17]</sup>.the effect of the inclusion complex on diuresis was accompanied by marked increase in urinary  $\text{Na}^+$  and  $\text{Cl}^-$ , their effect on electrolyte excretion was significant. HCT group showed a decrease in urinary potassium secretion, this result joined the study on the diuretic effect of crude extracts of *Carissa edulis* in rats or reference group received 10 mg of the drug HCT <sup>[18]</sup>. While the inclusion complex displayed a pronounced kaliuretic activity. Diuretics modulate the volume and composition of body fluids in variety of clinical conditions like hypertension, heart failure, nephritic syndrome and cirrhosis. In primary hypertension, sodium is considered an important external factor. Numerous studies have shown the adverse effects of increased sodium uptake on arterial blood pressure <sup>[19]</sup>. Previous investigations of diuretic agents have found it advantageous to pretreat or prime the test animal with various fluids. As diuretics are employed clinically in the treatment of oedema, it would seem to be most important to demonstrate effectiveness in the presence of electrolyte and water. Thus, excess water and electrolyte was given to stimulate oedema <sup>[18]</sup> Diuresis has two components: increase in urine volume (water excretion) and a net loss of solutes (i.e. electrolytes) in the urine <sup>[20]</sup>. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The reference drug, thiazide diuretics inhibit the  $\text{Na}^+/\text{Cl}^-$  symporter (co-transporter system) in the distal convoluted tubule, by competing for the  $\text{Cl}^-$  binding site, and increasing the excretion of  $\text{Na}^+$  and  $\text{Cl}^-$  <sup>[20]</sup>.Indirectly, the diuretic action of hydrochlorothiazide reduces plasma volume, with consequent increase in urinary potassium loss, plasma renin activity, aldosterone secretion and decrease in serum potassium . while the furosemide, increases urine output and urinary excretion of sodium by inhibiting  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  symporter (co-transporter system) in the thick ascending limb of the Loop of Henley <sup>[20]</sup>. Compared the three groups, control,HCTalone and HCD- $\gamma$ CD inslucion complex, results suggested that  $\gamma$ CD increased the pharmacological effect of the HCT. This improvement was related to the increase in the solubility and bioavailability of HCT <sup>[13]</sup>.This result seems to be in line with the study reported by Maria Arlete Silva Pires et al. who's studied the biological diuretic effect of HCT- $\beta$ CD, the results showed that the diuretic effect of inclusion complexes was



higher and significantly different in comparison to HCT alone and control. Data showed also statistically different increase of the amounts of electrolyte  $\text{Na}^+$  in the HCT- $\beta$ CD group in comparison to the HCT and control groups 24 hours after administration <sup>[21]</sup>. Increased solubility profiles of CD were previously reported in the literature for many drugs <sup>[22-25]</sup>, including diuretics such as spironolactone <sup>[26]</sup>.

## CONCLUSION

In conclusion, the present study demonstrated that the diuretic activity of HCT- $\gamma$ CD complex was increased compared to the HCT alone and control group. This improvement can be explained by the enhanced solubility and bioavailability of HCT by its inclusion with  $\gamma$ -cyclodextrine. The present study indicates that HCT- $\gamma$ CD complex is a potential candidate as a diuretic agent

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