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Effect of Aqueous Extract of *Mimosa invisa* Mart Ex Colla (Fabaceae) and Some Diuretic Substances on Kidney Function of Wistar Rats

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ABSTRACT

Pharmacopoeia traditional plant, *Mimosa invisa* (Fabaceae) is used in the treatment of various pathologies including diabetes and high blood pressure and has diuretic properties. This study objective was to assess its effect on renal function in Wistar rats strain. The study showed that the aqueous extract of *Mimosa invisa* (Miv), at a dose of 20 mg/kg B.W., 24 hours after its administration to rats, causes an increase in urinary urea excretion and a decrease in urea in the blood. The ratio of these two urea increases in treated rats with *Mimosa invisa*. The extract leads to a decrease of creatinemia and increases urinary creatinine and creatinine clearance. These effects are similar to those of reference diuretics. The aqueous extract of *Mimosa invisa* is therefore a diuretic. It does not appear to have a detrimental effect on renal function, but rather has a beneficial effect on glomerular filtration, which would justify its use in traditional medicine in the treatment of several pathologies, in particular with high blood pressure.

INTRODUCTION

Africa has a significant diversity of medicinal plants that treat a variety of diseases (Dibong et al., 2011). Among them, is *Mimosa invisa* Mart ex. Colla (Fabaceae), a plant used in traditional medicine in Africa to treat various pathologies including diabetes and hypertension (Mansroi, 2011). Thus, study has been maked to evaluate the toxicity of the fresh juice of *Mimosa invisa* (Usha 2009). The potential hypoglycemic effect of the aqueous extract of this plant was revealed by Manosroi (2011). The antihypertensive and diuretic effects have been demonstrated by Irie bi et *al.,* (2016). According to Naouaoui (2019), nephrotoxicity is one of the major undesirable effects of the use of medicinal plants, due to the physiological importance of the kidney and the diversity of plants that can affect it. Similarly, several studies have shown that prolonged diuretic use can lead to dehydration, acute tubular necrosis due to dehydration and electrolyte disorders (Naouaoui, 2019). Similarly, several studies have shown that prolonged use of diuretics can lead to dehydration, acute tubular necrosis in dehydration and electrolyte disorders (Naouaoui, 2019). In spite of its many traditional uses and in view of the many pharmacological potentialities already demonstrated, it is essential to verify the effects of the use of this plant on kidney function.

Therefore, this study assessed the effect of the aqueous extract of *Mimosa invisa* (Miv) on the kidney function of Wistar rat.

MATERIALS AND METHODS

Plant material

The plant material consists of the leafy twigs of *Mimosa invisa* Mart ex. Colla (Fabaceae). This plant was collected in Cocody (Abidjan, Ivory Cost) and identified at the National Floristic Center (CNF) of the Felix Houphouët-Boigny University (UFHB) compared to herbaria No. 7160 of December 14, 1963 of (late) Laurent Ake-Assi, Emeritus Professor of Botany, and No. 199 of June 18, 1992 of Emmanuelle Ake-Assi Doctor of Botany.

Animals

Rats of the species *Rattus norvegecus* (Murideae), of Wistar strain and of Musa genus, bred at the animal house of UFHB Biosciences Training and Research Unit (UFR) (Abidjan, Côte d'Ivoire) are used. These animals weigh between 100 and 120 g. They are fed at will with granules supplied by the *Ivograin*® Company of Abidjan and have free access to water

during rearing, which is done in cages at the animal house, at a temperature of 25 ± 2°C, with a humidity of 55-60% and a photoperiod of 12 hours (light/dark).

All experimental protocols were conducted in accordance with the European directive of November 24, 1986 (86/609/EEC) and the decree of April 19, 1988 (Anonymous, 1986) on the protection of laboratory animals.

Physiological solution

The physiological solution used is NaCl 9 ‰

Chemical substances

The chemicals used in this work are furosemide (Sanofi Aventis, France), hydrochlorothiazide (Novartis pharma, Switzerland) and spirolactone (Pfizer Holding, France).

Methods

Preparation of *Mimosa invisa* extract

For extraction, 200 g of fresh leafy twigs of *Mimosa invisa* (Fabaceae) are boiled for 45 minutes with 3 liters of distilled water. The resulting decoction is filtered 3 times on hydrophilic cotton, then twice on filter paper (Whatman n° 1 paper). The collected filtrates are dried in an oven (Vacutherm Vacuum Oven, France) at 50°C for 4 days. The dry extract obtained, which is in the form of an orange-yellow powder, is the aqueous extract of *Mimosa invisa* (Miv).

Experimental protocol

Twenty-five (25) rats were divided into 5 batches of 5 and acclimatized for 48 hours in metabolic cages. These animals are first fasted for 18 hours before the experiment, without water or food. After 18 h, the animals are each given distilled water at a rate of 50 ml/kg body weight (B.W.) orally, and then 0.5 ml of the test substances is injected into each animal intraperitoneally as follows: lot 1 receives NaCl 9 ‰ lot 2 receives 20 mg/kg (B.W.) orally, and 0.5 ml of the test substances is injected intraperitoneally as follows: lot 1 receives NaCl 9 ‰ lot 2 receives 20 mg/kg (B.W.) orally. C). of Miv; lot 3 receives 20 mg/kg (B.W.) of furosemide; lot 4 receives 20 mg/kg (B.W.) of hydrochlorothiazide and lot 5 receives 20 mg/kg (B.W.) of spirolactone. Finally, these animals are placed in metabolic cages for 24

hours. Their urine is collected and their blood is drawn for urea and creatinine determinations using the Cobas C311 automatic multi parameter analyzer (Roche Diagnostics, France). The determination of these parameters is done kinetics and colorimetric.

Determination of creatinine in blood and urine

Creatinine, in an alkaline solution, reacts with picrate to form a colored complex.

The determination of creatinine is done according to the method described by Jaffe (1886) and modified by Fabiny and Ertingshausen (1971). The reagent consists of 2 solutions, a picric acid solution and a sodium hydroxide solution. The reading wavelength is 492 nm.

Determination of urea in blood and urine

The enzymatic methodology for the determination of urea is based on the reaction described by Talke and Schubert (1695) and modified by Tiffany, et *al.*, (1972) according to which the concentration of urea is proportional to the change in absorbance over a given period of time. The reagent consists of urease, glutamate dehydrogenase (GLDH) and nicotinamide adenine dinucleotide in reduced form (NADH) and acetoglutarate (a-CG).

The initial decrease in optical density at 340 nm is proportional to the urea concentration of the sample (Bretaudiere, 1976).

Determination of creatinine clearance

Creatinine clearance is determined according to the following equation:

$$Cl_{crea} = \frac{(Creat_{ur} \times V_{ur})}{Crea_{pl} \times V_{ur}}$$

Cl_{crea}: Creatinine Clearance **$Creat_{ur}$:** Creatinine in urine

V_{ur}: volume of urea obtained after 24 hours (µL) **Duration:** 24 hours (i.e. 1440 minutes)

Statistical analysis and graphics

The results are analyzed by the variance analysis (ANOVA) of the Tukey-Kramer multiple comparison test. $p< 0.05$ is considered significant. All values are presented as mean ± standard error on the mean.

The graphs are plotted by GraphPad *Prism* 5 software (San Diego CA, USA).

RESULTS

1- Effects of aqueous extract of *Mimosa invisa* (Miv) and reference diuretic substances on the urea level in rats

In control rats, 24 h after receiving NaCl 9 ‰ the urinary urea rate is 6.71 ± 0.37 g/L. 24 h after the animal treatments, rats each receiving 20 mg/kg B.W. of *Mimosa invisa* aqueous extract (Miv), furosemide or hydrochlorothiazide had urinary urea rates of 9.27 ± 0.43 g/L, 9.65 ± 0.68 g/L and 9.56 ± 0.69 g/L, respectively; increases of 38.15%, 43.8% and 42.47%, significant ($p < 0.05$) compared to controls. In those treated with spirolactone at 20 mg/kg B.W., the measured of urinary urea rate was 12.46 ± 0.57 g/L; a significant ($p < 0.001$) increase of 85.69% over controls (Figure 1).

In serum, on the other hand, urea rate drops significantly when the rats are treated with the extract or substances (Figure 2). The decreases are 20.88%, 22.15%, 22.15% and 34.66% respectively with Miv (0.25 ± 0.012 g/L), furosemide (0.246 ± 0.015 g/L), spirolactone (0.246 ± 0, 014 g/L) ($p < 0.05$) and hydrochlorothiazide (0.212 ± 0.02 g/L) ($p < 0.01$) compared to control rats which is 0.316 ± 0.009 g/L.

The ratio of urinary urea/plasma urea increased significantly ($p < 0.001$). In treated rats with Miv, spirolactone, hydrochlorothiazide and furosemide, the ratios were 37.08, 51.91, 43.45 and 40.20, respectively, representing increases of 71.34%, 139.87%, 100.78% and 85.76%, respectively, compared with controls with a ratio of 21.64 (Figure 3).

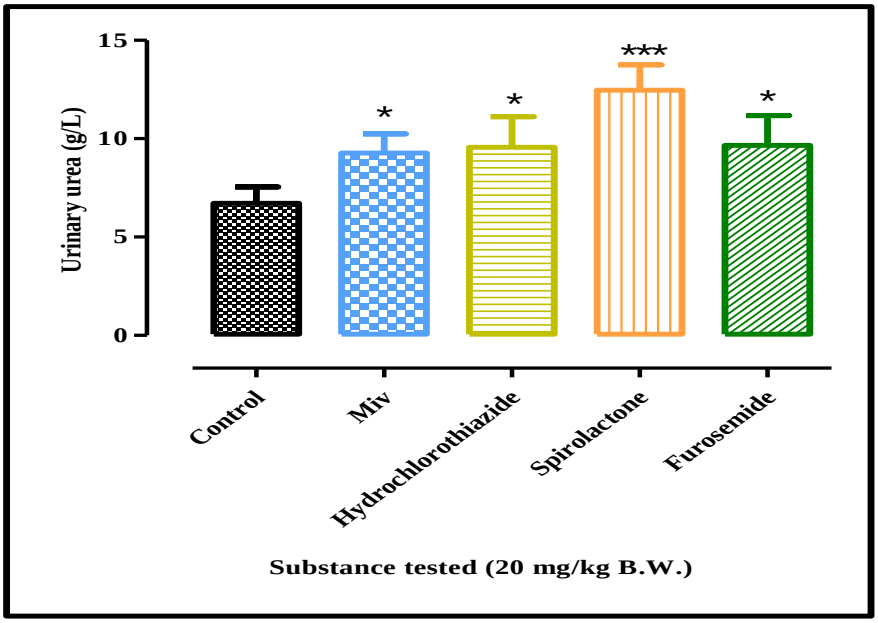

Figure No. 1: Urinary urea rates in rats treated with aqueous extract of *Mimosa invisa* (Miv) or various diuretic substances

n = 5; *: p < 0.05; ***: p < 0.001 compared to the control

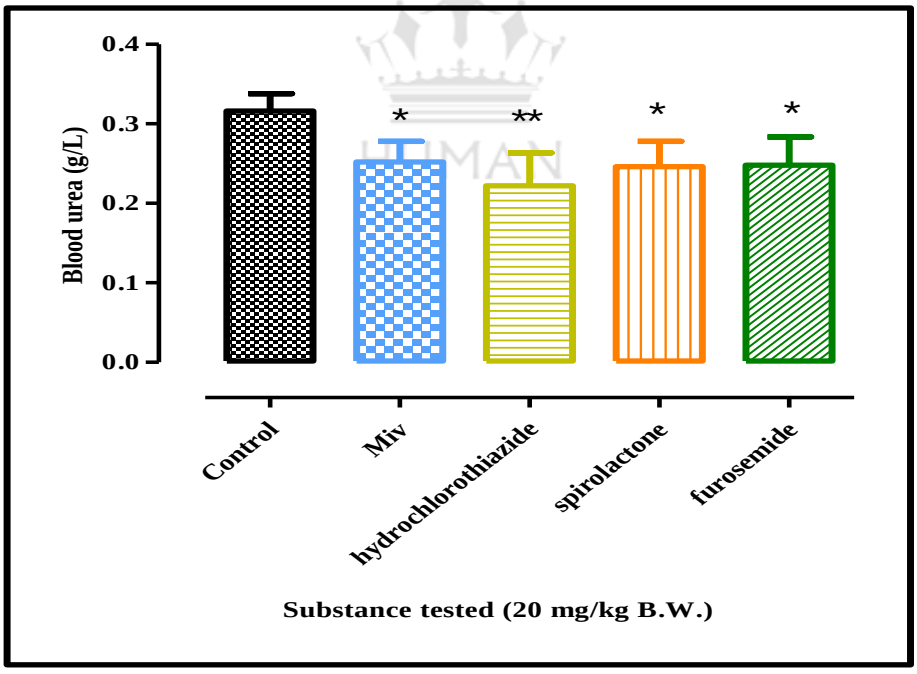

Figure No. 2: Urea rate in the blood in rats treated with aqueous extract of *Mimosa invisa* (Miv) or various diuretic substances

n = 5; *: p <0.05; **: p <0.01 compared to the control

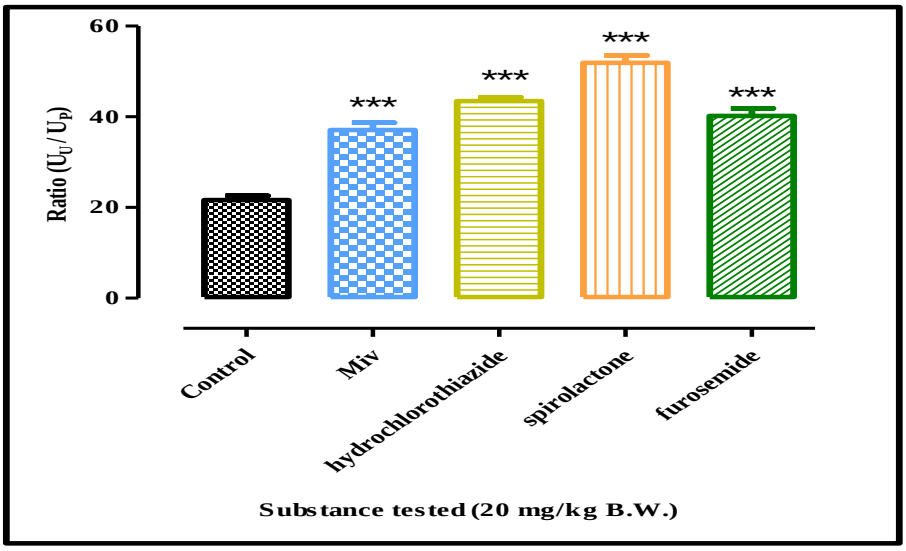

Figure No. 3: Urinary urea/plasma urea ratio in rats treated with aqueous extract of *Mimosa invisa* (Miv) or various diuretic substances

n = 5; ***: p <0.001 compared to the control

2- Effects of the aqueous extract of *Mimosa invisa* (Miv) and reference diuretic substances on creatinine in rats

Urinary creatinine levels increased only slightly ($p < 0.05$) in treated rats with Miv, hydrochlorothiazide and furosemide and very significantly ($p < 0.001$) with spirolactone, compared to controls. The creatinine rate with control rats is 3.08 ± 0.22 mg/L. In treated rats, creatinine rates were 6.26 ± 0.39 mg/L in the presence of Miv, 6.44 ± 0.74 mg/L with hydrochlorothiazide, 6.16 ± 0.70 mg/L with furosemide and 16.03 ± 0.99 mg/L with spirolactone; increases in creatinine of 103.24%, 109.09%, 100% and 420.45%, respectively, compared to controls (Figure 4).

In the serum of treated rats with Miv, spirolactone, hydrochlorothiazide and furosemide, the decreases in creatinine rates of 25.43%, 27.23%, 27.98% and 28.42% ($p < 0.01$), respectively, were obtained compared to controls. Blood creatinine rates in treated rats were 9.94 ± 0.57 mg/L in the presence of Miv, 9.7 ± 0.3 mg/L with hydrochlorothiazide, 9.6 ± 0.63 mg/L with spirolactone and 10.38 ± 0.48 mg/L with furosemide, compared to 13.33 ± 0.20 mg/L for control rats (Figure 5).

The serum urea/creatinine ratio increased significantly ($p < 0.05$) in treated rats with Miv and spirolactone by 9.53% and 8.88%, respectively, compared to controls. In those treated with

hydrochlorothiazide and furosemide, this ratio does not vary significantly ($p > 0.05$) from controls. In fact, this ratio is 25.15 ± 0.57 with Miv, 22.68 ± 0.40 in the presence of hydrochlorothiazide, 25 ± 0.32 with spirolactone and 23.12 ± 0.32 with furosemide, while it is 22.96 ± 0.55 for the control (Figure 6).

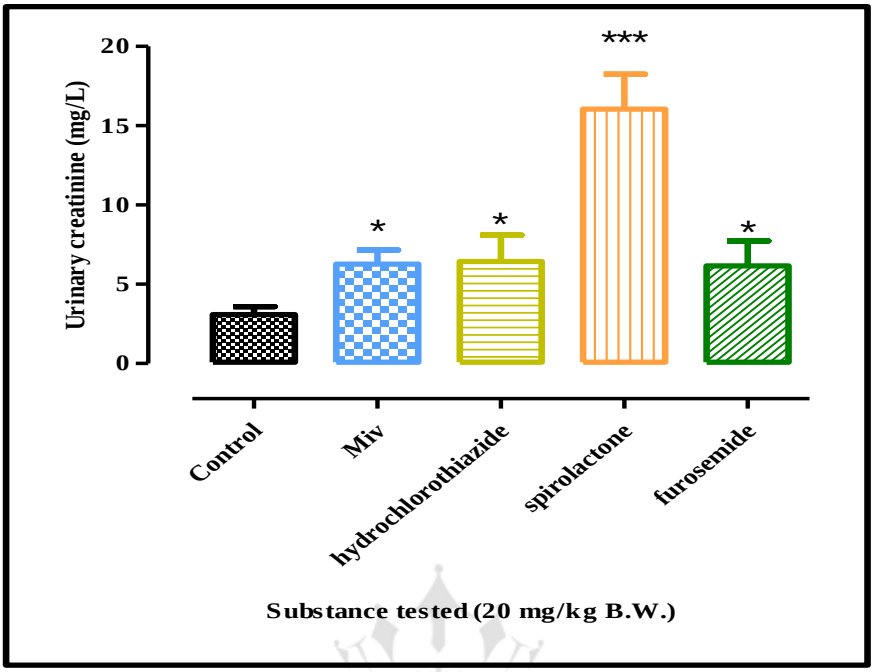

Figure No. 4: Urinary creatinine rate in rats treated with aqueous extract of *Mimosa invisa* (Miv) or various diuretic substances

n = 5; *: p <0.05; ***: p <0.001 compared to the control

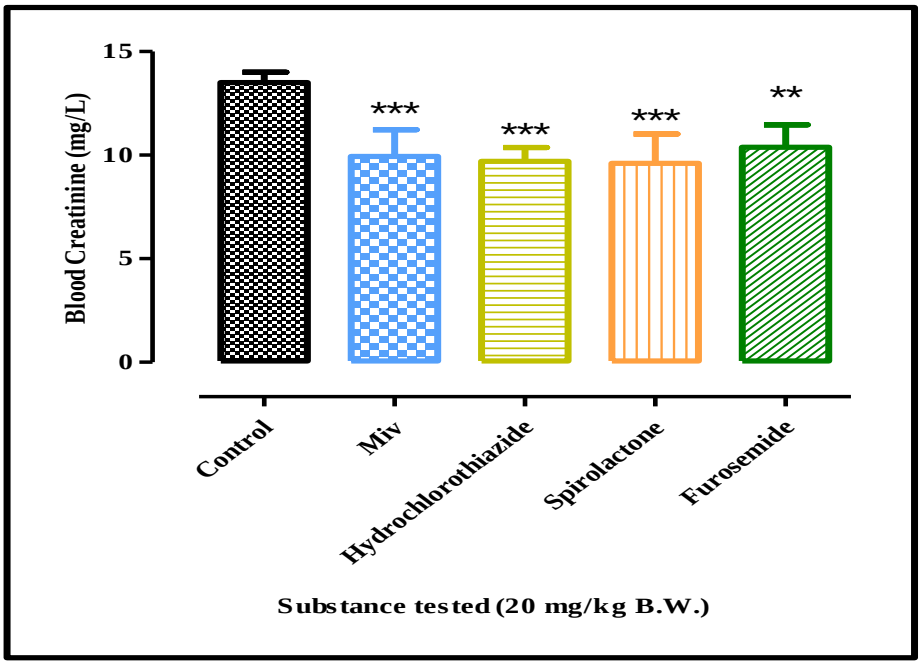

Figure No. 5: Plasma creatinine rate in rats treated with aqueous extract of *Mimosa invisa* (Miv) or different diuretic substances

n = 5; **: p <0.01; ***: p <0.001 compared to the control

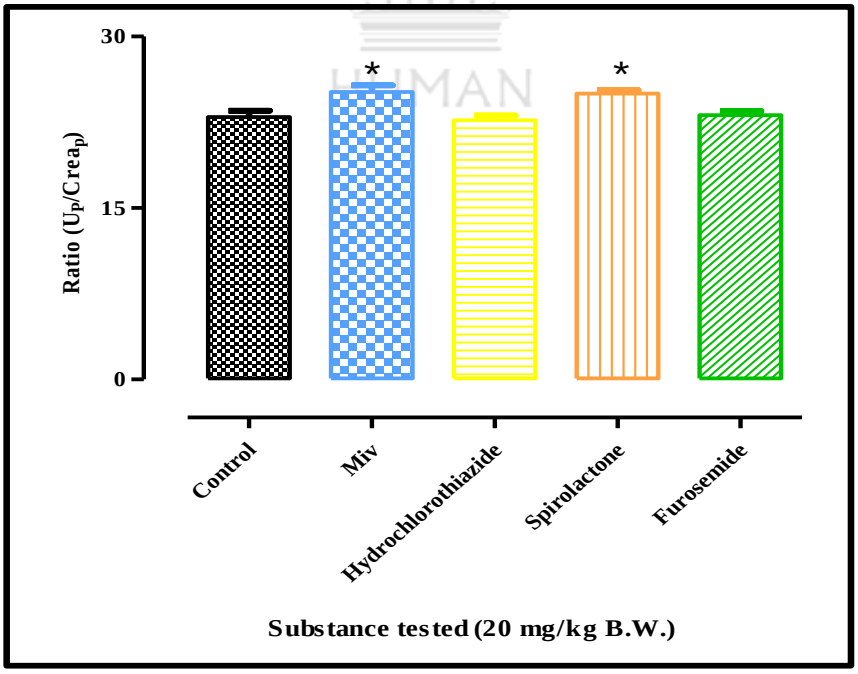

Figure No. 6: Serum urea to creatinine ratio in rats treated with aqueous extract *of Mimosa invisa* (Miv) or various diuretic substances

n = 5: *: p <0.05 compared to the control

3- Effects of the aqueous extract of *Mimosa invisa* (Miv) and reference diuretic substances on clearance in rats

Creatinine clearance increases significantly ($p < 0.001$) with Miv (18.05 ± 0.51 µL/min), hydrochlorothiazide (19.98 ± 0.39 µL/min), spirolactone (44.23 ± 1.21 µL/min) and furosemide (18.56 ± 0.41 µL/min) compared to the control (1.65 ± 0.03 µL/min) (Figure 7).

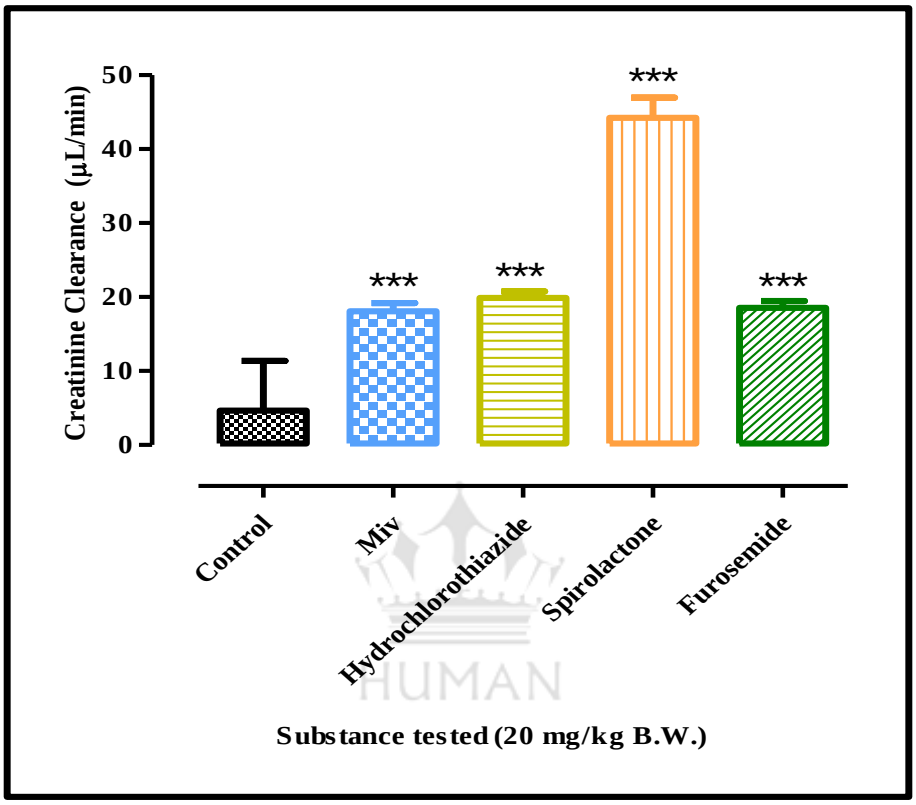

Figure No. 7: Creatinine clearance in rats treated with aqueous extract of *Mimosa invisa* (Miv) or different antidiuretic substances

n = 5; ***: $p < 0.001$ compared to the control

DISCUSSION

The capacity of kidney function in this study was verified by assessing urinary and serum urea and creatinine rates, as well as urinary urea to plasma urea ratio, creatinine clearance and serum urea to creatinine ratio. All of these are clinical markers of renal function (Froissart et *al.*, 2008).

This study shows that Miv does not increase serum creatinine rates, suggesting that the aqueous extract of *Mimosa invisa (*Miv), does not cause glomerular and/or tubular damage.

Better still, Miv shows a decrease in Blood creatinine and an increase in urinary rates, which would suggest a beneficial effect of Miv in the treatment of some nephropathies. Indeed, creatinine is strictly eliminated by the kidneys. Its dosage is used in the evaluation of kidney function and more particularly in the estimation of glomerular filtration rate (Charriere et *al.*, 2009).

Creatinine is a nitrogenous molecule produced by the body through the catabolism of creatine, which is a protein compound contained in muscular tissue. Creatinine is a physiologically inert molecule (Andrew et *al.*, 1988). This means that it is neither metabolized nor used in any way in organism. An increase of the creatinine concentration in the blood is an indication of kidney dysfunction. Data on its concentration in the blood and urine can be used to estimate the glomerular filtration level. Thus, an increase in serum creatinine concentration by a substance means that it causes glomerular and/or tubular damage (Frank, 1992).

Our results also indicate that Miv decreases serum urea rates and increases urinary rates, which shows that Miv does not cause glomerular damage, but rather may be able to boost kidney function if there is underlying nephropathy. Indeed, urea represents the main form of nitrogen elimination synthesized during the catabolism of proteins by the liver. It is one of the first markers used to measure glomerular filtration level. Urea passes into the nephrons regardless of its concentration in the blood; it is a non-threshold substance (Chanton and Paniel, 1966). This molecule is freely filtered in the glomerulus but is reabsorbed in the proximal and distal tubular. A high rate generally indicates glomerular damage or hepatotoxicity, which is common with many toxins (Frank, 1992).

This study also shows that the urinary urea/plasma urea ratio increases considerably with a value which is well above 10, meaning that the use of Miv for therapeutic purposes would not induce renal failure. Indeed, this ratio, when it is below 10, indicates organic renal failure (Brunet et *al.*, 2007). A reduction in the urinary urea/plasma urea ratio with an increase in blood urea more than creatinine would have oriented towards acute tubular necrosis (Bankir, 2012).

The kidneys filter the blood to form urine through which certain metabolites in the blood are excreted. This process begins with glomerular filtration. To assess it, concepts such as creatinine clearance are used. Urinary clearance is the ratio between the urinary output of a substance and its concentration in the blood. Thus, if the substance clearance is high, the

purifying power of the substance is high. Since creatinine is only eliminated by kidney filtration, measuring the creatinine clearance allows the filtration rate of the kidneys to be assessed. A decrease in creatinine clearance generally indicates kidney failure (Cockcroft, 1976; Levey et *al.*, 1999; Cheyron et *al.*, 2008). Creatinine clearance increases significantly in rats after Miv injection. This is in favour of the beneficial effect of Miv on glomerular filtration.

The effects of Miv on creatinine clearance are similar to those of furosemide, which is a Henle's loop diuretic (Anderson et *al.*, 1971). This suggested that Miv would act by a mechanism similar to that of furosemide on glomerular filtration. Thus, like the loop diuretics, Miv would act by blocking the sodium-potassium-chlorine transporter at the ascending Henle loop, thus reducing the demand for O_2, thereby minimizing the effects of renal vasoconstriction-induced ischemia observed in tubular necrosis. In addition, by increasing urine flow, it would also decrease tubular obstruction created by necrotic tubular cells (Kellum, 1998).

Also, Miv in increasing glomerular filtration could have a likely beneficial effect in the treatment of acute renal failure. Indeed, according to Sautan et *al.*, (2001), acute renal failure is defined as a rapid decline in glomerular filtration and characterized by a loss of vascular self-regulation, resulting in a renal plasma flow entirely dependent on renal perfusion pressure, and thus a particular sensitivity of the renal parenchyma to variations of the blood pressure. This would confirm the antihypertensive effects of Miv on the one hand and, on the other hand, its probable beneficial effect in the treatment of acute kidney failure.

The serum urea/creatinine ratio increases significantly 24 hours after an intraperitoneal injection of Miv. This ratio reflects variations in urea and creatinine inputs during renal filtration. Low urea-creatinine ratios may be associated with reduced protein intake or starvation, acute tubular necrosis or severe liver disease (Barton et *al.*, 2007). Thus, Miv does not alter renal or liver functions.

CONCLUSION

This study highlights the effect of the aqueous extract of *Mimosa invisa* (Miv) on kidney function in rats 24 hours after administration. This extract leads to a significant decrease in serum urea and an increase in the urinary urea and the urinary urea/plasma urea ratio. It also significantly lowers creatinine levels and increases urinary creatinine, clearance and serum

urea to plasma urea ratio. The results suggest that this extract does not alter kidney function when used in the traditional treatment of various conditions. This extract leads to an increase in glomerular filtration and its effects are similar to those of some loop diuretics, thus confirming that Miv would act as a diuretic, which could justify its use in traditional medicine in high blood pressure treatment.

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