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Hypotensive Activity of Tridax Procubens hydro ethanolic extract: Roles of transport of sodium and potassium in Rat Wistar

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ABSTRACT

In Africa in general and Benin in particular the population is increasingly confronted with several pathologies such as hypertension. Even though modern medicine is well developed, this population uses traditional medicine because of its low purchasing power. But the lack of mastery of the dosage and its underlying effects remain archaic parameters and deserves special attention from the scientific world from which we have chosen to reflect on Tridax procumbens in order to come up with scientific arguments favorable to the promotion of the «Plant species. The present work was carried out in order to evaluate the effect of hydroethanolic extracts on the plasma and urinary concentrations of potassium, sodium and chloride ions in Wistar rats. After the preparation of the extracts and their gavage, the extraction yield was evaluated, the level of the K +, Na +, and Cl- ions was assayed in three batches of three male Wistar rats. Lots 2 and 3 respectively received doses of 300 mg / kg and 500 mg / kg of single-dose PC in one day, respectively. Control batch 1 received distilled water in place of the extracts. At the same time, they were placed in cages with urine sampling, for the measurement of diuresis. The extraction yield by the hydroethanolic solvent was 16.75%. Extracts at a dose of 300 mg / kg of PC induced hyperkalaemia, with no effect on serum sodium and chloraemia within two hours of administration of the extracts. The diuresis is so insignificant that we can understand that hyperkalaemia, induced bradycardia arising from hyperpolarization of the cells of the pace maker. Its hypotensive property is therefore confirmed.

Keywords: Tridax procumbens, Ionic channels, Hyperkalemia, hypernatremia, Hyperchloremia, Diuresis, Hyperpolarization.

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INTRODUCTION

Since the creation of the world, humanity has been confronted with various forms of illness and different origins which it must resist in order to perpetuate itself and to perpetuate itself in time. Some of them are transmissible microbial and other non-transmissible (NCD) or chronic, among which we have genetic diseases and nutritional diseases because of poor diet or unbalanced diet. Contrary to the prevailing view, NCDs do not primarily affect high-income populations. Not communicable diseases, once the diseases of developed and industrialized countries, will constitute an important public health problem in sub-Saharan Africa after 2020 (WHO 2000). As a result, if the NCD epidemic is not acted on aggressively in the most heavily affected countries and communities, their impact will continue to grow, undermining the global goal of poverty reduction. In 2005, according to WHO, 60% of deaths were due to NCDs. In Benin, 80% of deaths due to NCDs occurred in developing countries. During the current epidemiological transition, deaths from infectious diseases, Childbirth and under nutrition are expected to decrease by 3% in the next decade, whereas deaths due to non communicable diseases should (WHO), 2005. Of the 57 million deaths worldwide in 2008, 36 million, or 63%, were due to NCDs, diabetes, cardiovascular diseases, cancer and chronic respiratory diseases. In African countries, NCDs are progressing rapidly and by 2030 they are expected to be a more common cause of death than communicable, maternal, perinatal and nutritional diseases. Thus, they tend to supplant infections and malnutrition (PRONANU, 2004). Cardiovascular disease, as a major cause of hospital mortality (TAMBWE M., et al., 1995) and certain cardiovascular risk factors (hypertension, obesity, diabetes mellitus, stress, alcohol and tobacco abuse) Epidemic-like prevalence rate in the workplace (NKOY, 2002). Type 2 diabetes is very often associated with high blood pressure, obesity and dyslipidemia. These elements characterize the metabolic syndrome (or syndrome X), an identified risk factor for heart disease (CVD), stroke and peripheral arterial disease. Management of the hypertensive patient requires ongoing monitoring and life-long treatment, expensive in hospitals, involving the combination of several therapies (Deteix et al., 2005). In sub-Saharan Africa, the prevalence of hypertension varies between 10 and 15% with a peak in South Africa. This alarming picture has prompted WHO / AFRO to classify the disease as a priority disease in Africa (Trapsida, 2003). In diabetics, achieving normal blood glucose and normal blood pressure are not enough. Intensive treatment of the lipid balance of type 2 diabetes remains the best way to prevent cardiovascular disease. As a result, most countries in sub-Saharan Africa do not have a formal organized system for the management of diabetes at the primary level. The high cost

of treating diseases through modern medicine is a major problem in Africa. To address these health problems, African populations are turning more towards traditional medicine than to modern medicine Natural resources have been an important source of medicinal recipes for centuries. Plants are one of the most used resources in traditional medicine. Indeed, the use of plants to treat diseases and other functional disorders is as old as mankind. Traditional medicine provides solutions for the treatment of several conditions including bacterial, fungal infections, malaria, diabetes, high blood pressure, sickle cell anemia and opportunistic infections contracted by people living with HIV / AIDS (Sofowora, 1993).). Thus, the World Health Organization (WHO) promotes the use of traditional medicinal recipes when evidence of efficacy, efficiency and safety is demonstrated (Sofowora, 1993). It is within this framework that health professional decision-makers struggle with issues of safety, efficacy, quality, availability, preservation and future development of this type of health care (WHO, 2002). The importance of plant use is greater in developing countries where traditional medicine is often the first and only recourse of the poor. In Africa and Asia, 80% of the population continues to use traditional medicines rather than modern synthetic molecules for first aid. The popularity of traditional medicine in developing countries is explained by the availability of plant resources used and the relatively affordable cost of medicinal receipts. Moreover, the efficacy of these recipes in the treatment of several pathologies and the absence of side effects make them more interesting than modern synthetic molecules (Ali and Ramachandran, 2001). But the lack of mastery of the dosage and its underlying effects remain archaic parameters and deserves special attention from the scientific world. Hence, we have chosen to carry out a scientific reflection on the "Effects of the Tridax procumbens hydroethanol extract on the plasma and urinary concentrations of Na +, K +, and Cl- ions in Wistar rats" especially since recent work by GUINRA A., 2015, confirms that Tridax procumbens is a hypoglycemic plant in diabetics; Pathology whose hypertension is a corollary. Other authors have also reported its hypotensive and bradycardiac properties (Diwan et al., 1989, Ravikumar et al., 2005). What are the effects of Tridax procumbens Hydro-Ethanol Extract on plasma and urine concentrations of Na +, K + ions in Wistar rats?

MATERIALS AND METHOD

Vegetable material

The leaves and stems of Tridax procumbens were harvested on the Abomey-Calavi campus during the short dry season on August 18, 2016. After their identification by the National Herbarium Laboratory of Benin at the University of Abomey- Calavi (see certificate in appendix) samples of

leaves and stems were cleaned and then dried at room temperature in the shade laboratory to better conserve molecules sensitive to heat and light. They were then crushed, pulverized and bottled.

Animal equipment

The experimental animals are male Wistar rats. All animals are of sanitary status EOPS (Exempt from Specific Pathogenic Organism). Upon receipt, the rats were randomly placed in groups of three (03) in standard cages for a five (05) day acclimation period before being used in the various experiments. During this period, the animals had free access to food and water and were kept in the laboratory of the Laboratory of Bio membranes and Cell Signaling at the University of Abomey-Calavi at a constant temperature of +/- 2) ° C. Then they were marked and weighed.

Methods of study

Extraction method

In order to obtain the total hydroethanol extract, 150 g * 3 or 450 g of the dried plant material (leafy stem powder) were weighed and then mixed with a volume of 500 mL of the solvent in a proportion of 50% (V / V). The mixture is left under magnetic stirring for 24 hours at room temperature. The solution obtained is then filtered on a filter paper (Wattman No. 1 with a diameter of 0.16 mm) on a Büchner device under vacuum. The filtrate was recovered and the operation was repeated 3 times (ie 72 hours of extraction in total). The total volume of the filtrate is concentrated under vacuum in an oven at a temperature of 40 ° C. under a reduced pressure. The recovered, weighed, labeled dry extract is stored at + 4 ° C until use. The yield "r" is calculated according to the following formula: r = (m2 / m1) * 100 with m1 = mass of dry matter (powder) and m2 = mass of the extract.

Methods of experimental study

The administration of the extracts is carried out orally (Per Os) in a single dose as follows: We have three batches of (03) rats. The first batch of control rat R1 to which extracts are not administered (control). A second batch of rat R2 which receives the extracts at the dose of 300mg / kg of body weight. A fina l batch of rats R3 was gavaged with a solution containing the extracts at a dose of 500 mg / kg body weight.

The principle is to administer the ethanolic extracts of Tridax procumbens to wistar rats (male) weighing between 100g and 140g and having the same food intake, orally and at various doses. These animals do not receive any other drug treatment during the experimental period outside the extract. The rats are randomly assigned to 03 batches, and the batches are then administered to the batches, respectively, per kg of body weight (see annex for the administration plan). At the beginning of the experiment, each of the rats in each batch is weighed in order to find the average

weight and the effective dose of extract to be administered is calculated. Lot 1 is the control lot. The rats of this lot will receive 01 mL of distilled water. At lot 2, the animals will receive 300 mg / kg body weight of the ethanolic extract of Tridax procumbens and the animals of lot 3 will receive 500 mg / kg body weight of the same extract. The various solutions will be administered orally with a syringe provided with a probe. Before feeding, the animals were fasted two hours earlier. Blood is taken from the three batches of rats. After gavage, the blood is also taken twice in a time interval of 2 hours to see the effect of the extracts on the levels of plasma Na +, Cl- and K + ions. On the other hand, we take a urine sample every two hours per batch, three times and the volume of urine per representative rat is measured every two hours. The urines conditioned in sterile dry test tubes are then sent to the analytical laboratory for the determination of ions, in particular Na + ions (Natriuresis).

Blood sample

The blood sample is taken according to the experimental protocol used by WOUETOLA (2014). Puncture of the retro-orbital sinus was performed without anesthesia. The animal is held with one hand in lateral decubitus, and held by the skin of the neck. The pressure of the thumb on the neck, behind the angle of the jaw, allows compression of the jugular vein, and therefore venous stasis towards the head, favoring the filling of the retro-orbital sinus. By making a slight traction on the upper eyelid with the index finger, we create an exophthalmos facilitating the taking of blood by means of hematocrit tube heparinized or not. The end of the tube is slowly introduced into the lateral angle of the eye. Progression through the tissues is facilitated by printing a small pipette rotation. As soon as the venous plexus is reached, the blood springs into the perorbital space and ascends by capillary action in the tube. The volume of blood collected is 0.5 to 2 ml. Before the tube is removed, the compression is released and the bleeding ceases spontaneously when the ocular pressure normalizes. The recovered blood is used for the determination of the various biochemical parameters. The blood is recovered in haemolysis tubes without anticoagulant and centrifuged at 3000 rpm for 15 min at a temperature of 4 ° C. After the centrifugation of the blood, the serum obtained is stored in eppendorf tubes at a temperature of -4 ° C. for the determination of the various ions.

RESULTS AND DISCUSSION

Calculation of hydroethanol extraction yield

The yield is calculated using the formula:

R= Mass of the extract (m2) / Mass of the bark powder (m1)× 100

Gold m2 = 75.41 g and m1 = 150 g × 3 = 450 g therefore R = (75.41 g / 450 g) × 100 = 16.75%.

Table 1 Yield obtained after extraction and physical characteristics of the extract

Extract	Yield in %	Color	Aspect
Macerated / ethanol-water	16,75	Dark Green	Pasty

The yield of 16.75% obtained for the hydroethanol extraction is low but close to that obtained by GUINRA Alimatou which is 17.075% in 2016. Nevertheless, this yield is higher than that of KOUKOUI et al in 2015 which Was 9.92%. Also, Ganju et al., In 2012 demonstrated that ethanolic and aqueous extracts manage to extract as much secondary metabolites as possible. Other authors in 2013 have shown that any solvent associated with water extracts most of the secondary metabolites contained in the plant.

The proximity of our results to those of GUINRA Alimatou., 2015 can be explained by the fact that the harvest is carried out on the same site of the University of Abomey-Calavi and this in the same period or the same season.

Ionic assays performed on blood samples (Blood plasma).

The purpose of this section is to follow the evolution of the mean concentrations (in mEq / L) of K +, Na + and Cl-plasma ions over time, ie before gavage of the extract T0, two hours after the gavage of the extract at T1 and then four hours after the gavage of the extract at T2 respectively at the doses of 300 mg / Kg of PC and 500 mg / Kg of PC of hydroethanol extracts of the leaves and stems of Tridax procumbens and to make sense of it.

Evolution and discussion of the level of potassium (K +) ions during treatment.

The measurements made enabled us to obtain the results which are summarized as follows:

Table 2: Variation of potassium in normal control rats treated with the hydroethanolic extract of the leaves and stems of Tridax procumbens.

K^+			
Time Lots	T_0 (before gavage)	T_1 (02 hours after gavage)	T_2 (04 hours after gavage)
sample	4,72 ±1,42	4,35±0,10	4,89±0,27
Lot treated with 300 mg / kg body weight.	4,24±0,05	6,643±3,46	4,51±0,94
Lot treated with 500 mg / kg body weight.	5,12±0,19	5,38±0,56	4,96±0,49

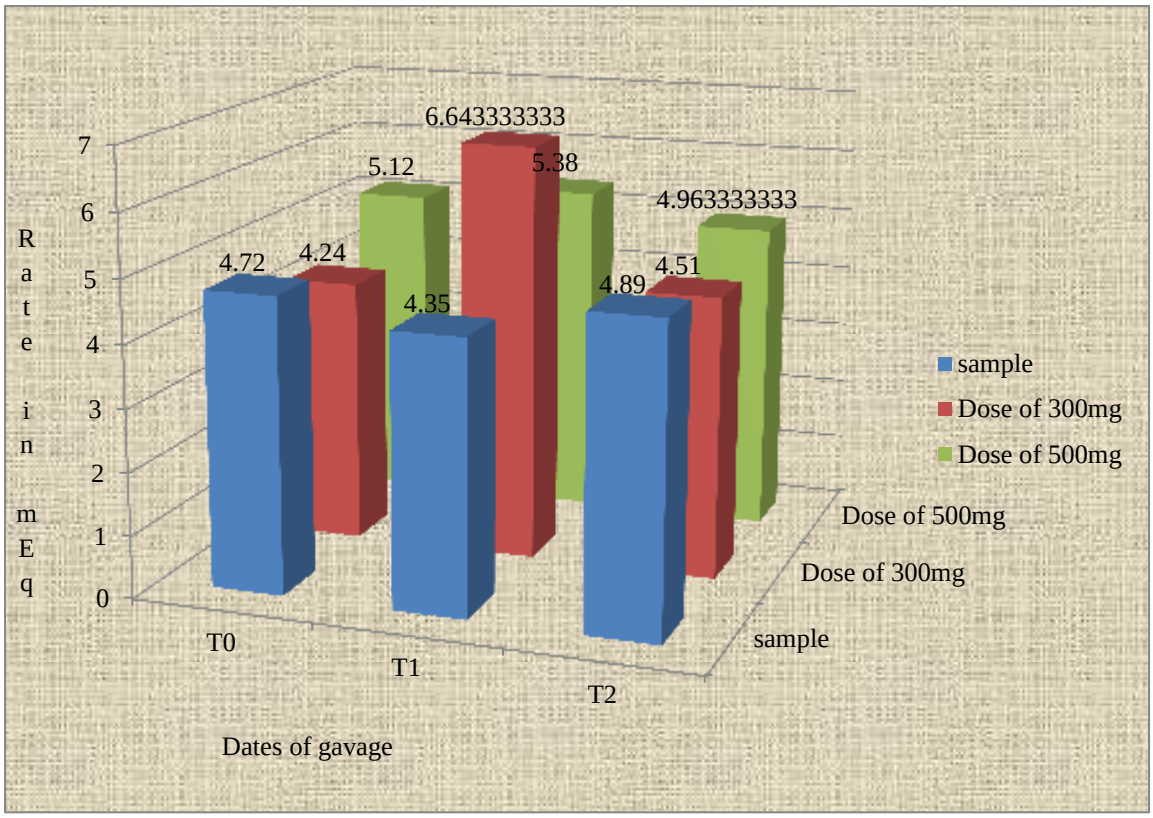

Figure 1: Evolution of the K + level in the normal control rats and those treated with the hydroethanol extract of the leaves and stems of Tridax procumbens.

We observed that in controls gavaged with 1 mL of distilled water, the plasma K + level fluctuated (decreased after 2 hours before increasing again after 4 hours) between 4.72 and 4.89 mEq / L, but in animals treated with 300 mg / kg of PC, although oscillating between 4.24 and 4.5 mEq / L, the K + level experienced a dizzy increase two hours after the gavage of the extracts of Tridax procumbens, Before decreasing after four hours. A similar increase in K + is observed in animals treated with the 500 mg / kg BW dose except that the increase is less than with the dose of 300 mg / kg of PC at the second hour. It can thus be understood that after two hours, the extracts provide a supplement of K + which causes a rapid rise in the plasma level of the K + ions. But after four hours the rates decrease because the organism probably mobilizes the hydro-electrolyte regulation system of potassium ions that restores balance. We can also understand that the most effective dose is the dose of 300 mg / kg of PC as GUINRA Alimatou, 2015 for the biochemical parameters of blood glucose, cholesterol and triglyceride levels. Moreover, it is reported that the aerial part of the plant is rich in potassium ion and that the plant possesses a hypotensive property (Salahdeen 2004 et al., Ravikumar et al., 2005). However, the role of potassium ions in regulating blood pressure is to cause bradycardia by inducing hyperpolarization of the cardiomyopathy. Therefore,

the hypotensive effect of the extracts is due to the addition of potassium ions. This allows us to validate our hypothesis.

Evolution and discussion of sodium ions (Na +) during treatment.

Plasma sodium ions yielded the results in Table 7.

Table 3: Change in sodium levels in normal control rats treated with the hydro-ethanol extract of the leaves and stems of Tridax procumbens.

Na+			
Time Lots	T_0 (before gavage)	T_1 (02 hours after gavage)	T_2 (04 hours after gavage)
sample	139,6±3,41	139,6±2,62	135,73±2,49
Lot treated with 300 mg / kg body weight.	140,35±1,90	140,03±5,70	136,15±0,49
Lot treated with 500 mg / kg body weight.	142,86±3,85	138,1±5,60	136,63±2,75

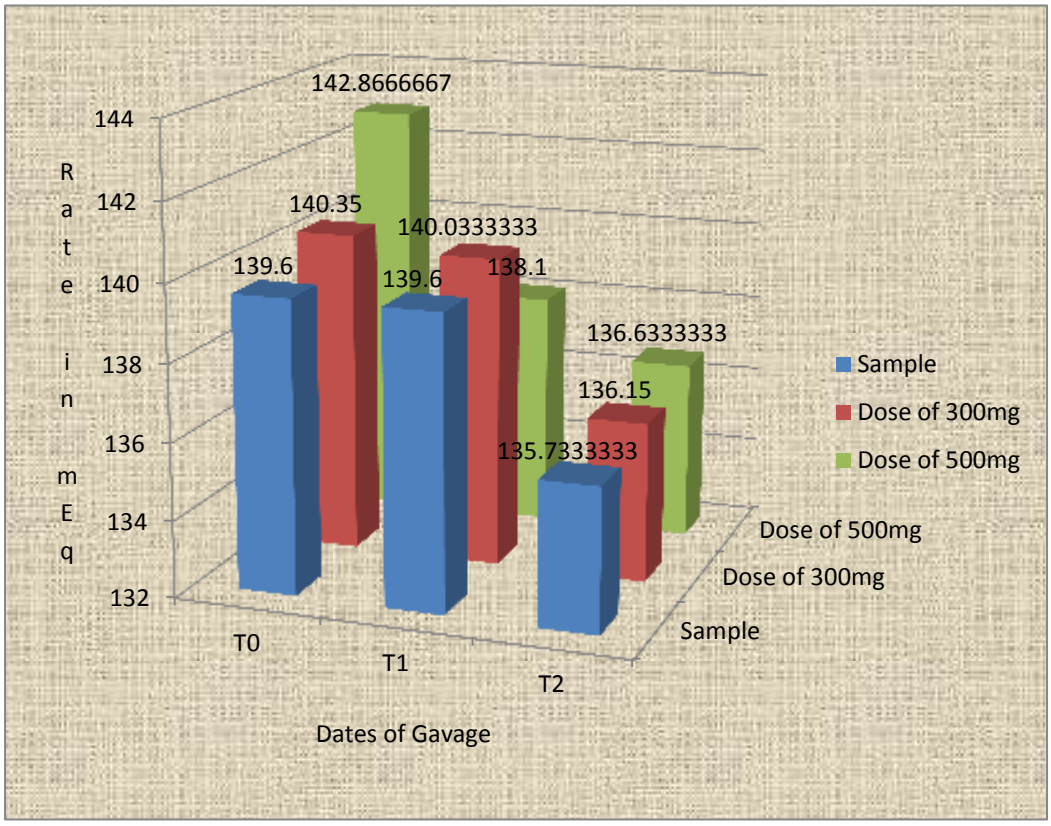

Figure 2: Evolution of the Na + level in the normal control rats and those treated with the hydroethanol extract of the leaves and stems of Tridax procumbens.

In animals treated with the 300 mg / Kg PC dose, after two hours, the serum nematoplasty did not vary significantly if it remained almost constant compared to the pre-gavage rate but slightly higher than Among the witnesses. This means that there is an inadequate intake of sodium ion by

the extracts that caused a non-significant variation. After four hours, the sodium level in the same animals dropped and returned to the same level as in the controls. This demonstrates the existence of a system of regulation or mobilization of sodium ions for physiological organic uses. With the animals treated at the dose of 500 mg / kg of PC, two hours after force-feeding, as at four after force-feeding, the serum level decreased almost to the same level as in the controls. Thus, it can be retained that the hydroethanolic extracts of Tridax procumbens do not cause a significant variation in the level of sodium ions in the blood.

Evolution and discussion of the chloride (Cl-) ion level during the treatment.

The measurements made on the blood of the rats gave the results of Table 8:

Table 4: Chloride variation in normal control rats treated with the hydroethanol extract of the leaves and stems of Tridax procumbens.

Cl-			
Time Lots	T_0 (before gavage)	T_1 (02 hours after gavage)	T_2 (04 hours after gavage)
sample	105,93±2,33	106,5±1,96	104,83±1,02
Lot treated with 300 mg / kg body weight.	102,3±1,55	108,93±7,97	99,65±7,28
Lot treated with 500 mg / kg body weight.	107,56±2,82	107,43±4,66	105,76±2,68

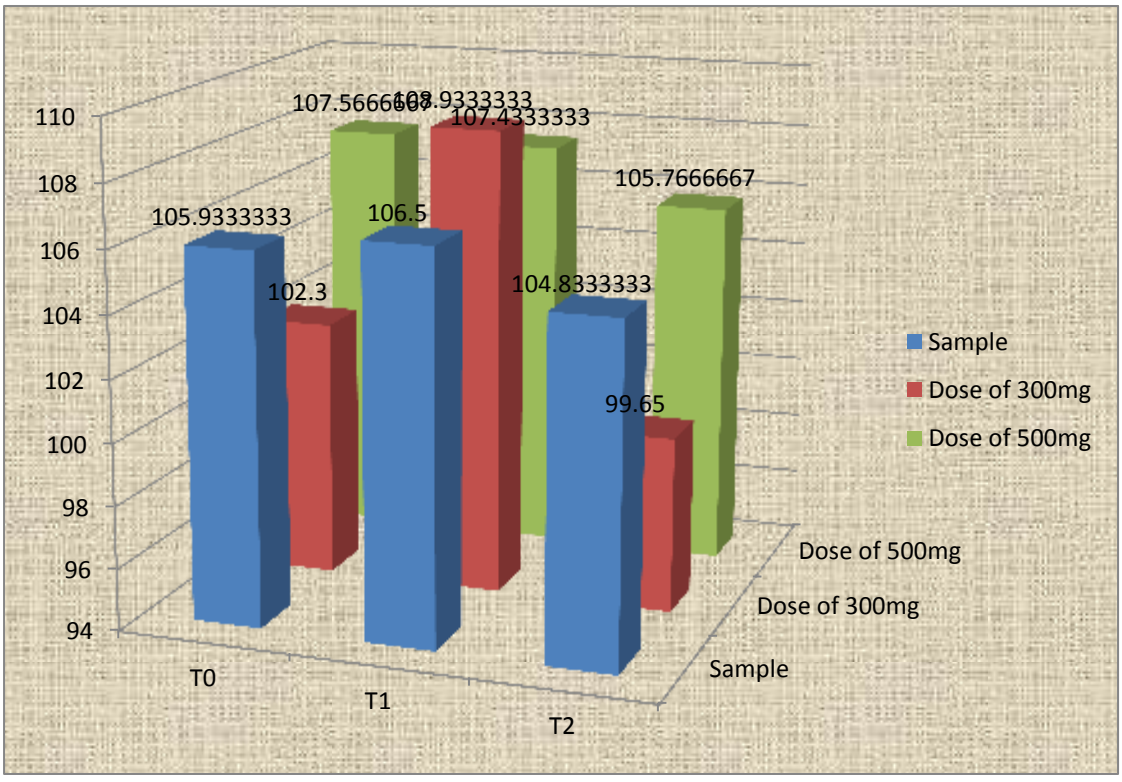

Figure 11: Evolution of the Cl- level in the normal control rats and those treated with the

hydroethanol extract of the leaves and stems of Tridax procumbens.

As regards chloride ions, the ion level increases slightly after two hours after the glucagon-blotting of hydro-ethanolic extracts in rats treated with 300 mg / kg of PC while in rats treated with Dose of 500 mg / kg of PC chloramic acid did not increase significantly compared to controls such as what was read at time T0 before gavage. In addition, after four hours, the concentration of chloride ion decreased in rats treated with 300 mg / kg of PC as well as in rats treated with 500 mg / kg of PC but with a more remarkable fall in the first than the second. It appears therefore that the extracts at the dose of 300 mg / kg of PC induce a slight increase in the level of chloride ions in the blood, two hours after the gavage of the rats. And the fall in rates, after four hours, is not significant. The variations of the chlorae are comparable to those of the natremia.

Measurement of diuresis and discussion

The measurements of the volume of urine collected for the 3 rats of the control batch treated with 01 ml of distilled were done. It is apparent from the reading of these tables that the treated rats did not urinate, as did the control rats. It is deduced that the extracts of Tridax procumbens have no effect on diuresis in rats. This invalidates our hypothesis. It follows that the hydro-ethanolic extracts of Tridax procumbens do not cause hypotension via active diuresis as do many hypotensive drugs.

CONCLUSION

Nowadays, in order to face their numerous health problems, the populations of Africa, in general those of Benin in particular, because of poverty, use a range of medicinal plants. Among these plants, several have been the subject of scientific study, with respect to Hypertension. it appeared in our study that the experimentally administered rats had a very low diuresis within two or four hours after the administration of the Tethaxax Hydroethanol extracts. This shows that the extracts did not cause renal elimination of NaCl and invalidates our hypothesis. We can conclude that treatment with the hydro-ethanolic extracts of Tridax procumbens induces hyperkalaemia, confirming our hypothesis, after two hours without significant effect on serum sodium and diuresis. This demonstrates that the hypotensive effect reported on the extract of Tridax procumbens is not due to a decrease in serum sodium and blood volume via active diuresis as do many hypotensive drugs; which invalidates our hypothesis. The hypotensive effect of the extract of the aerial parts of Tridax procumbens is therefore linked to its very high content of potassium ions. Hyperkalemia leads to bradycardia and a drop in blood pressure, by inducing hyperpolarization of the cardiac muscle and thus opening the channels to potassium ions.

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