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EXPERIMENTAL STUDY OF THE ALKANIZING EFFECT OF ETHANOL AND AQUEOUS EXTRACTS OF *CURCUMA LONGA* L. ON URINARY ACIDITY IN WISTAR RATS

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ABSTRACT
The development of traditional therapies as pharmacological drugs to treat acidosis is becoming increasingly worrying. A number of medicinal plants have shown promising activity. The aim of this study was to evaluate the alkaline effect of ethanolic and aqueous extracts of *Curcuma longa* L. in wistar rats. For this purpose, we carried out an ethanolic extraction on the one hand with *Curcuma longa* L. powder and 90°C ethanol and an aqueous extraction on the other hand with *Curcuma longa* powder and distilled water. We then proceeded to force-feed 16 wistar rats in 6 batches. The administration of the extracts was carried out every morning over 7 days at two doses (0.4 and 0.8mg/kg body weight) after direct pH measurement with strips. At the end of this extraction, we obtained a yield of 8% in aqueous extraction and 6.5% in ethanolic extraction. The results showed that the administration of 0.4mg/kg and 0.8mg/kg body weight of aqueous and ethanolic extracts of *Curcuma longa L.* powder in wistar rats had a significant alkaline effect on the urinary pH of the latter. Although all extracts reached their maximum action at D7, the ethanolic extract at 0.8mg/kg body weight was more effective. The ethanolic extract was more effective than the aqueous extract. The maximum action of all the extracts was pH 9. All in all, *Curcuma longa* L. has a significant alkaline effect. This study could be repeated on a large sample of wistar rats in order to confirm or refute the results of the present study. These studies could address the toxicity of the latter mainly on the liver and kidneys of wistar rats.

KEYWORDS: *Curcuma longa* L., alkalizing effect, wistar rats, acidosis, Benin.

INTRODUCTION
Metabolic acidosis is a disorder frequently found in hospitals, particularly in emergency and intensive care units, but also in internal medicine and visceral surgery (Throssell et *al*., 1995; Martin et *al.*, 2005). Acidosis, although not really considered a disease in itself, becomes pathological when it remains permanent and at a higher than normal rate (Chevrolet et *al*., 2002). An evolution towards too much acidity in the body leads to health problems and takes us away from an ideal acid-base balance (Kraut et *al*., 2001; Rose et *al*, 2002). This tendency to tissue acidosis is increased: during any illness, especially chronic illness, because of the tissue anoxia it causes; during stress, which increases sympathicotonia, a source of muscular tension that produces lactic acid; in infections; when taking medication, such as aspirin derivatives (salicylic acid is a strong carboxylic acid), morphine, non-steroidal anti-inflammatory drugs (Rodriguez et *al*., 2002). Nicoletta et *al*, 2004; Kraut et *al*, 2005; Stucker, et *al*, 2007). This acidosis also leads to the severe malaise described by Rizk et al. in 1999 in children. The development of traditional therapies as pharmacological drugs to treat these acidoses is becoming increasingly worrying. A number of medicinal plants have shown promising activity in China and Japan (Stickel et *al.*, 2007).

Curcuma longa L. is a rhizomatous perennial herbaceous plant that belongs to the Zingiberaceae family, native to South Asia and commonly known as turmeric. In Malaysia, commonly known as Kunyit, turmeric is a popular ingredient in the preparation of culinary dishes. In addition, it is used as a herbal remedy due to the widespread belief that the plant has medicinal properties. In traditional medicine, the rhizome juice of *Curcuma longa* L. is used in the treatment of many diseases such

as antihelminthics, asthma, gonorrhoea and urinary diseases, and its essential oil is used in the treatment of carminatives, stomach and tonic (Phansawan et *al*., 2007). In traditional medicine, several plants and herbs have been used experimentally to treat liver disorders, including cirrhosis of the liver, (Alshawsh et *al.*, 2011; Kadir et *al*., 2011). Turmeric longa L. has antioxidant (Maizura et *al.*, 2011), anti-tumour (Kunnumakkara et *al*., 2007), antimicrobial (Kim et *al*., 2005), anti-inflammatory (Kohli et *al*., 2005), healing (Panchatcharam et *al.*, 2006) and gastroprotective (Miriyala et *al.*, 2007) activities. Previous studies have also shown that the aqueous extract of Curcuma longa had hepatoprotective activity against carbon tetrachloride toxicity (Sengupta et *al*.,2011).

However, very few studies have evaluated its alkaline effect in wistar rats. It is within this framework that we proposed to study its alkaline activity through this work which we titled: Experimental study of the alkanising effect of ethanolic and aqueous extracts of turmeric longa on urinary acidity in wistar rats. To carry out this study, we adopted a four-part plan. The first is devoted to generalities; the second to the study framework and the materials and methods; in the third part we presented the results and discussions; and finally the conclusion, perspectives and suggestions.

MATERIAL AND METHODS

Materials

Plant material

The plant material consists of Curcuma longa L powder. *Curcuma longa* was harvested in April 2018 in Djougou by a team of environmentalists. It is dried under the shelter in the room for 3 months and 11 days, then finely ground. The powder obtained is then stored in a plastic bottle with a lid to avoid contamination. This packaged powder was used as plant material for our experiment.

Animal material

We have six batches for our work, including two batches of two wistar rats each representing the control batches (negative and positive) and four experimental batches of three wistar rats each. These animals are subjected to sub-chronic force-feeding for 7 days.

Laboratory consumables used

These were: pH reading scale, gloves, decontamination solution, toilet paper, tube rack, etc. The pH was measured by the pH meter strips.

Methods

Preparation of the ethanolic extract of *Curcuma longa* powder

To obtain the ethanolic extract of *Curcuma longa* L powder, 400g of powder was macerated in 4000 ml of ethanol under continuous stirring for 72 hours before being filtered using cotton wool and Joseph's filter paper. The filtrate was then passed through the rotary evaporator at 45°C and a pasty deposit was obtained. The whole was then put in an oven at 45°C to evaporate the solvent and obtain the dry extract. It is these extracts that will be used to prepare the tested solutions. The yield is determined by the ratio of the weight of the dry extract after evaporation to the weight of the dry plant matter used for extraction multiplied by 100 (MEDANE et *al*., 2012).

Preparation of the aqueous extract of *Curcuma longa* L. powder

400 grams of *Curcuma longa* powder were weighed and macerated in 4000 ml of distilled water on a Bioblock scientific Fisher stuart shaker for 72 hours at room temperature. The homogenate obtained was filtered (03) times on cotton wool and two (02) times on Whatman N° 1 paper (Qualitative Circles 150 mm Cat N° 1001 150). The filtrate was dried at a temperature of 45°C in the oven to isolate the solvent (water) from the extract. The extract thus obtained represents the total aqueous extract ready for use and used to prepare the tested concentration ranges.

Administration of extracts

The extracts were administered to the animals by force-feeding. The technique consisted of immobilising the rat by holding it by the neck and vertically with its head up and tail down. A precise volume of solution was then taken from each batch using a syringe fitted to a probe and introduced into the animal's oesophagus and the solution was administered. The equipment consisted of: 1ml syringes, mouth probe, rinsing water, aqueous extract dissolved in distilled water, ethanolic extract dissolved in a 1/5 hydro-ethanol solution. Batch administration was carried out as follows:

- Batch 1 Negative control: the 05 rats are subjected to their normal diet in addition to 1ml of tap water per day and for the duration of the experiment;

- Batch 2 Positive control: the 05 rats are fed their normal diet in addition to 0.36ml of Cleanshield (reference food supplement at ph=14) per day for the duration of the experiment;

-Batch 3: the three (05) rats each received 0.4mg/kg body weight of aqueous extract per day in addition to their normal diet;

-Batch 4: the three (05) rats received each and every day, in addition to their normal diet, 0.8mg/kg body weight of aqueous extract;

-Batch 5: the three (05) rats received each and every day, in addition to their normal diet, 0.4mg/kg body weight of ethanolic extract;

-Batch 6: the three (05) rats received each and every day, in addition to their normal diet, 0.8mg/kg body weight of ethanolic extract; the administration of the extract was carried out every 7 days between 7am and 9am.

Table I: Summary of the batches of wistar rats for gavage.

Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
1ml of water	0.36ml of Cleanshield	0.4mg/kg extract aqueous	0.8mg/kg extract aqueous	0.4mg/kg extract ethanolic	0.8mg/kg extract ethanolic

Urine pH test

The test was carried out with a strip pH meter on the urine of the 16 rats every morning between 7 and 9 am. To obtain the urine, the rat was attacked by grabbing it by the back and placing it in the supine position. Automatically the rat emits a miction directly put in contact with the strip. The principle of this test is based on Ehrlich's reaction. The colour changes from yellow to dark blue, characteristic of alkalinization.

Procedure followed for the reliability of the test

For accurate and reliable results, do not compare the strip with the colour chart (the scale) before the strip is immersed in the urine. Therefore, after contacting the strip with the urine at the test area for no more than two seconds, avoiding excess urine on the strip, we turned the strip on its side and tapped once on a piece of absorbent material to remove any trace of urine as excessive urine on the strip can cause chemicals to interact between adjacent reagent plates. Then the colours of the reagent tablets were compared after 60 seconds with the colour reading scale on the vial label under good lighting. Finally, the strip was kept horizontal to avoid possible mixing of chemicals in case of excessive urine.

RESULTS AND DISCUSSIONS

Results

Evolution of pH in wistar rats Positive control after 7 days' feeding with cleanshield.

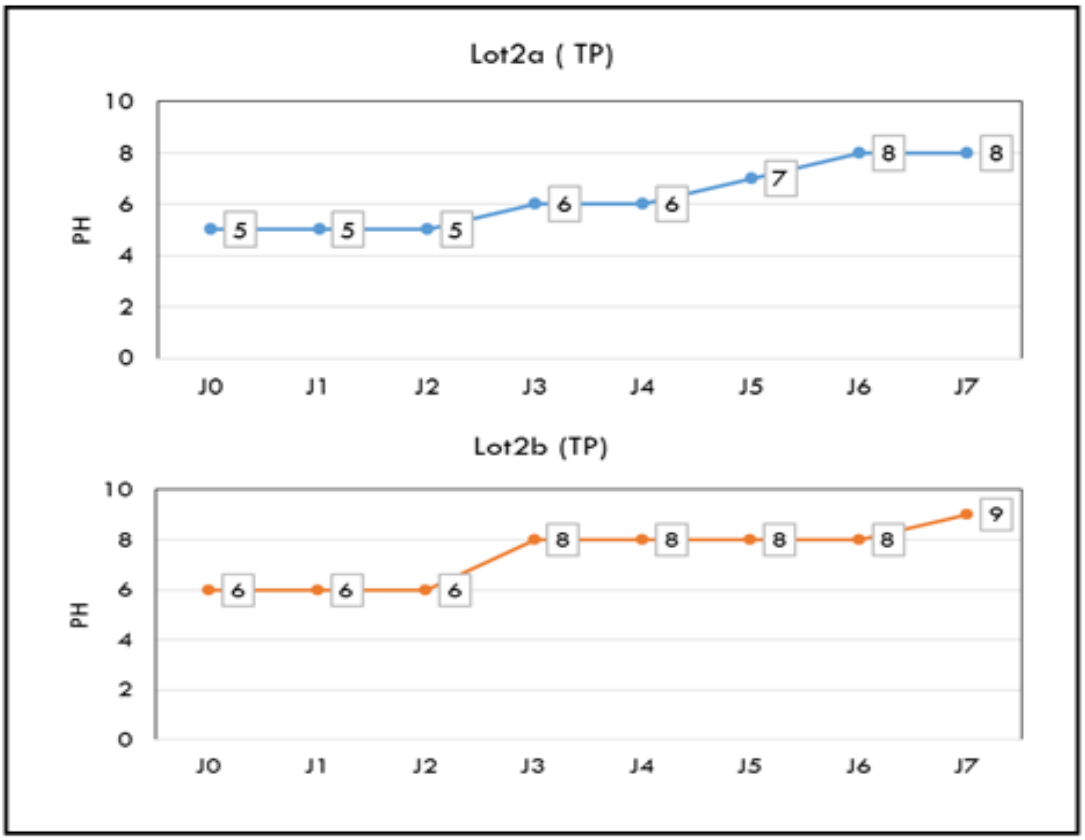

Figure 1: Evolution of pH in wistar rats Positive control after 7-day gavage with cleanshield.

We notice that until the 2nd day, the urinary pH of the two rats having received 0.36ml of cleanshield had always remained constant. From the 3rd day of gavage, an increase in pH was observed. Indeed, in the 1st rat (Lot2a), the pH constantly increased to reach a value of 8 on the 6th day, and remained stable until the 7th day. As for the second rat (Lot2b), after an increase of 2 units between the 2nd and 3rd day, the pH remained stable before reaching a value of 9 on the 7th day. The administration of 0.36ml of cleanshield for 7 days significantly influenced the variation of urine pH in wistar rats. pH change in wistar rats after gavage of 0.4 mg/kg aqueous extract for 7 days.

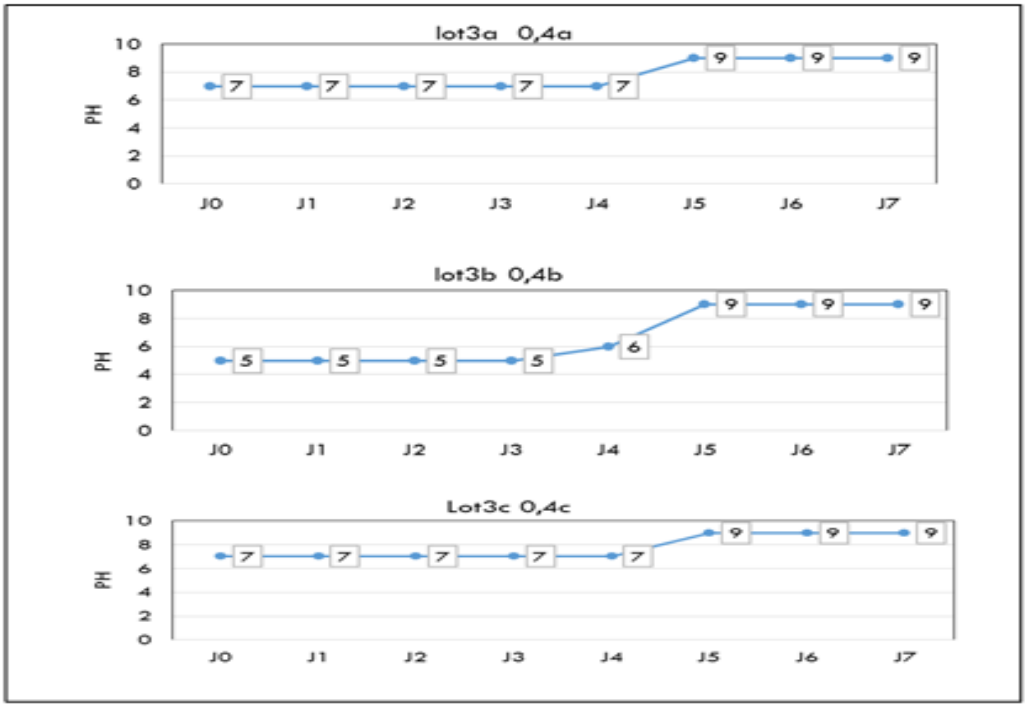

Figure 2: Evolution of pH in wistar rats after gavage of 0.4 mg/kg aqueous extract for 7 days.

The evolution of the urinary pH in Lot3a and Lot3c rats was identical. The administration of the aqueous extract of Curcuma longa powder only had an effect from the 4th day in these two rats. During the first 4 days of the experiment a urine pH of 7 was recorded in these two rats. The urine pH then increased to 9 on day 5. The urinary pH of these 2 rats did not experience any further changes until the end of the experiment. The 2nd rat (Lot 3b), just like the other two, had a urinary pH which underwent a 3-phase evolution. During the first phase, the pH remained constant at 5 (until 3 days). Then, between the 3rd and 5th day, the pH increased from 5 to 6 and then from 6 to 9. Finally, the urine pH remained stable until the end of the experiment.

The administration of the aqueous extract of *Curcuma longa* L. powder for 7 days significantly influenced the variation in urine pH.

Changes in pH in wistar rats after gavage of 0.8 mg/kg aqueous extract for 7 days

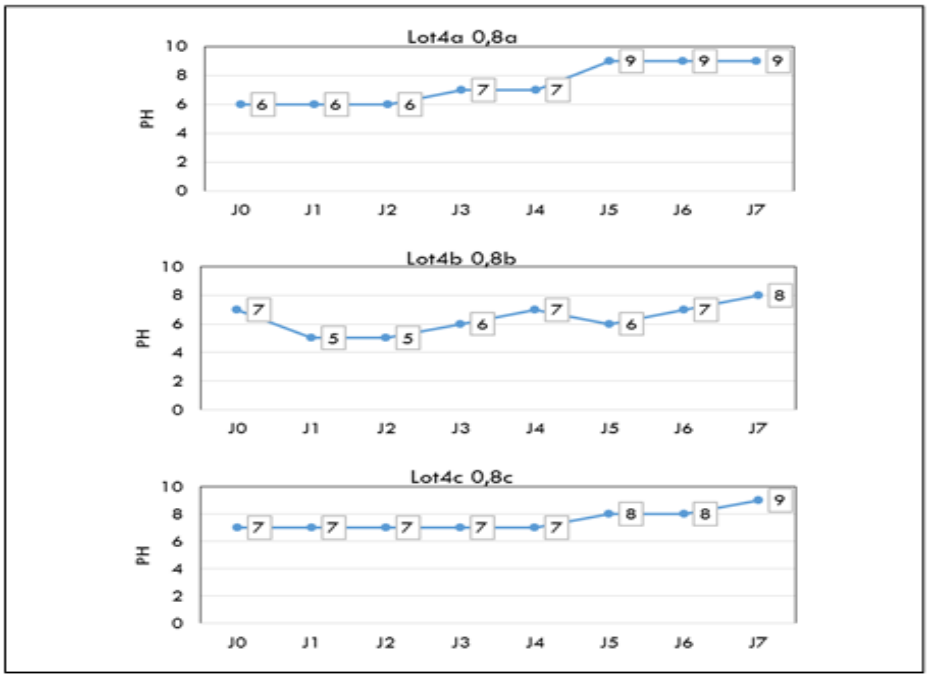

Figure 3: Evolution of pH in wistar rats after gavage of 0.8 mg/kg aqueous extract for 7 days.

Administration of 0.8mg/kg body weight of the aqueous extract of Curcuma longa powder in rats of lot 4 had an effect on the urinary pH of the rats. More specifically, rats Lo4a and Lot4c at the end of the 7 days of experiment reached a value of 9, while Lot4b rat on day 7 had a pH of 8.

Evolution of pH in wistar rats after gavage of 0.4 mg/kg of ethanolic extract for 7 days.

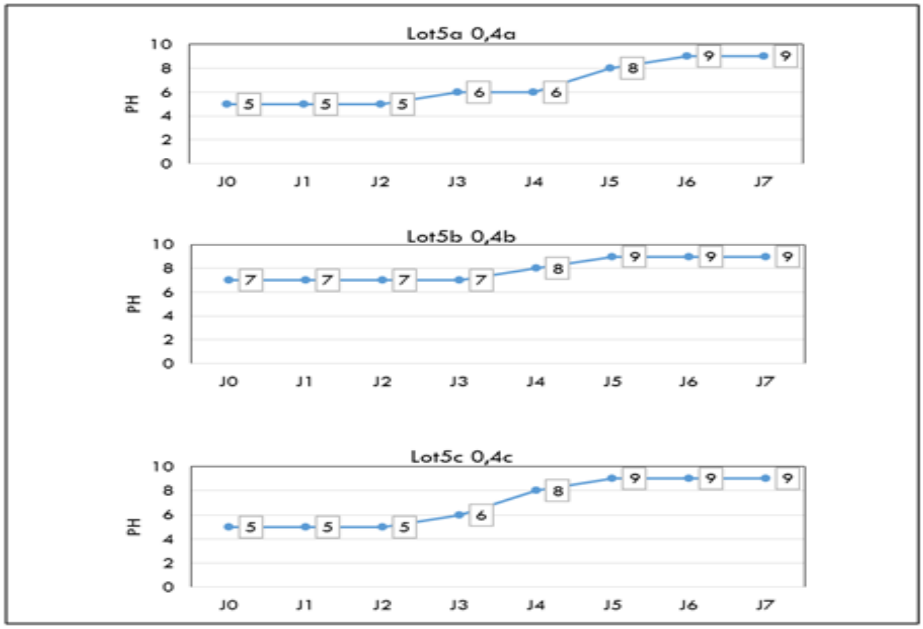

Figure 4: Evolution of pH in wistar rats after gavage of 0.4 mg/kg of ethanolic extract for 7 days.

The urinary pH values of rats given 0.4mg/kg body weight of the ethanolic extract of Curcuma longa powder showed a similar pattern. The urinary pH remained stable until day 3, before reaching a maximum value (pH9) 2 or 3 days before the end of the experiment.

Evolution of pH in wistar rats after gavage of 0.8 mg/kg ethanolic extract for 7 days.

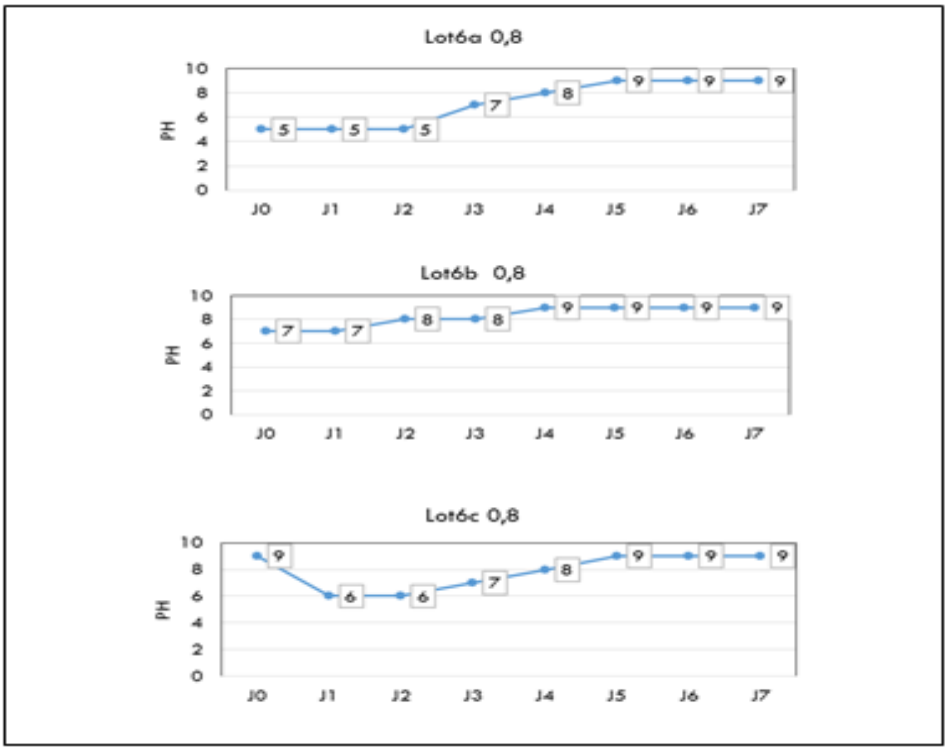

Figure 5: Evolution of pH in wistar rats after gavage of 0.8 mg/kg of ethanolic extract for 7 days.

At the end of the experiment, rats receiving 0.8mg/kg body weight of the ethanolic extract of *Curcuma longa* powder had a pH of 9 compared to 5, 7 and 9 respectively for rats lot6a, lot6b and lot6c at the beginning of the experiment. It should be noted that the urinary pH of the lot6c rat decreased by 3 units on day 1.

Determination of the dose and duration of action of aqueous and ethanolic extracts of *Curcuma longa* L.

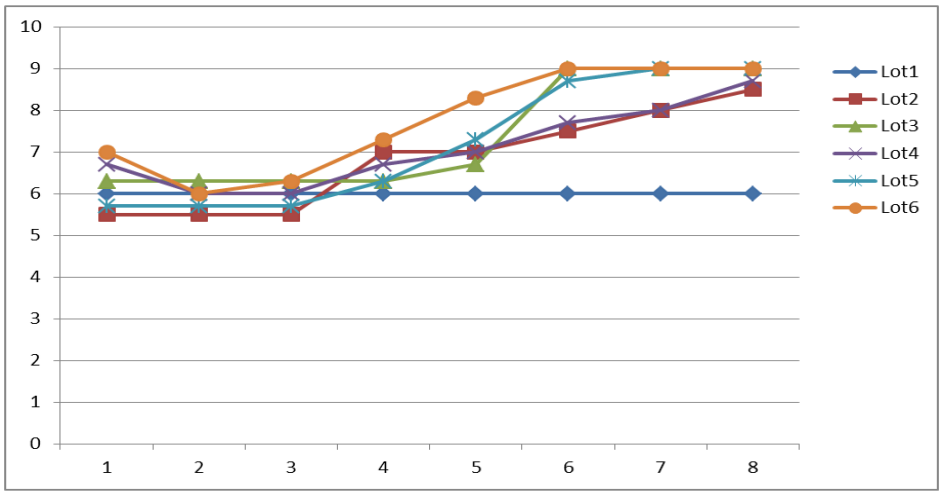

Figure 6: pH variations as a function of treatment duration.

From this figure, it can be seen that:

- The doses of aqueous and ethanolic extracts tested showed a satisfactory alkanizing effect over the 7 days of treatment;

- The ethanolic extract at a dose of 0.8 ml reached its alkaline effect first at D5;

- The alkanizing capacity of the aqueous and ethanolic extracts in this study was higher than that of the Positive Control;

- The two doses 0.4 and 0.8 mg/kg of aqueous extracts showed the same maximum alkaline effect at D6, whereas only the 0.8 mg/kg dose of ethanolic extract reached its optimum action on the same day. The 0.4 mg/kg body weight dose of ethanolic extract reached its maximum action at D7;

- The peak of action of the 0.8mg/kg dose of ethanolic extract was faster than the 0.8mg/kg dose of aqueous extract, D5 versus D6.

- The action peak of the 0.4mg/kg ethanolic extract dose was the same as that of the 0.4mg/kg aqueous extract dose at D 6.

DISCUSSION

The general objective of this study was to evaluate the alkaline effect of ethanolic and aqueous extracts of *Curcuma longa* L. in wistar rats. For this purpose we performed an ethanolic extraction with 90°C ethanol and an aqueous extraction with distilled water. After this extraction, we obtained a yield of 8% in aqueous extraction and 6.5% in ethanolic extraction. The yield of the aqueous extraction was slightly higher than that of the ethanolic extraction. The results showed that the administration of 0.4mg/kg and 0.8mg/kg body weight of aqueous and ethanolic extracts of *Curcuma longa* L. powder in wistar rats had a significant alkaline effect on the urinary pH of the latter. Testing at a dose lower than 0.4mg/kg could be considered since both doses of each of the extracts showed a higher alkanizing effect than the Positive Control composed of Cleanshield. Our search for articles evaluating the alkanizing effect of Curcuma longa L. extracts in scholargoogle and freefullpdf was unsuccessful. Therefore, we did not find any articles evaluating the alkanizing effect of *Curcuma longa* L. in wistar rats. However, studies have evaluated the effect of *Curcuma longa* L. and curcumin on the treatment of diseases that are also caused by metabolic acidosis. These diseases include: peptic ulcer, diabetes, cancer, urinary acidosis, urinary diseases, etc. The alkanizing effect of *Curcuma longa* L. obtained in this study would justify its effectiveness in the treatment of diseases caused by metabolic acidosis. Indeed, in traditional medicine, the rhizome juice of *Curcuma longa* L. is used in the treatment of many diseases such as anthelmintics, asthma, gonorrhoea and urinary diseases, and its essential oil is used in the treatment of carminatives, stomach and tonic [Phansawan et al, 2007]. In traditional medicine, several plants and herbs have been used experimentally to treat liver disorders, including cirrhosis of the liver [Alshawsh et *al*, 2011; Kadir et *al*, 2011]. Turmeric longa L. has antioxidant (Maizura et *al*., 2011), anti-tumour (Kunnumakkara et *al*., 2007), antimicrobial (Kim et *al.*, 2005), anti-inflammatory (Kohli et *al.*, 2005), healing (Panchatcharam et *al.*, 2006) and gastroprotective (Miriyala et *al.*, 2007) activities. Previous studies have also shown that the aqueous extract of *Curcuma longa* had hepatoprotective activity against carbon tetrachloride toxicity (Sengupta et *al*., 2011).

The maximum alkaline effect of the ethanol extracts was reached at D6 with the dose of 0.8mg/kg and at D7 with the dose of 0.4mg/kg. This difference is explained by the difference in the quantity of *Curcuma longa* L. per dose and demonstrates the effectiveness of the 0.8mg/kg dose compared to the 0.4mg/kg dose.

The ethanolic extract of *Curcuma longa* L. at the dose of 0.8gm/kg first reached the alkanizing effect at D5 while three (03) other doses tested showed their peak alkanizing effect at D6. The alkanizing activity of the 0.8mg/kg dose of ethanolic extract was obtained more quickly compared to that of the 0.8mg/kg dose of aqueous extract. These results show that the ethanolic extracts of *Curcuma longa* L. are more effective than the aqueous extracts. This could be explained by the difference in bioavailability of *Curcuma longa* L. according to the solvents used.

CONCLUSION

Within the framework of the evaluation of the therapeutic effects of medicinal plants, we were interested in the evaluation of the alkalizing effect of ethanolic and aqueous extracts of *Curcuma longa* L. in wistar rats. Following this extraction, we obtained a yield of 8% in aqueous extraction and 6.5% in ethanolic extraction. The results showed that the administration of 0.4mg/kg and 0.8mg/kg body weight of aqueous and ethanolic extracts of *Curcuma longa* L. powder in wistar rats had a significant alkaline effect on the urinary pH of the latter. Although all extracts reached their maximum action at D7, the ethanolic extract at 0.8mg/kg body weight was more effective. The ethanolic extract was more effective than the aqueous extract. The maximum action of all the extracts was pH 9. Other studies such as the evaluation of the toxicity of the extracts of *Curcuma longa* deserve to be carried out in order to deepen the results of this study.

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