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# STUDY OF THE ACUTE TOXICITY OF SEEDS AND PULPS OF *LAGENARIA SICERARIA* (MOL) STANDL USED IN TRADITIONAL NIGERIAN PHARMACOPEE

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## ABSTRACT

*Lagenaria siceraria*, a plant of the cucurbitaceae family, is widely used in traditional Nigerien medicine. The objective of this study was to evaluate the acute toxicity of aqueous extracts of the pulp and seeds of *Lagenaria siceraria* collected in the six (6) regions of Niger. For this purpose, the OECD protocol for the testing of chemicals was used. The results obtained show that all extracts induced changes in the general behaviour of the treated mice within the first two hours after administration. The aqueous extracts of seeds and pulp from the Tillabery and Zinder regions and the aqueous extract of pulp from the Maradi region showed no signs of toxicity. These extracts induced, at a dose of 300mg/kg body weight, a weight gain in mice following an increase in food intake. Conversely, extracts of seeds and pulps from the Tahoua, Niamey and Dosso regions and Maradi pulp extract caused disturbances in food and water intake as well as body weight. There were no deaths of mice during the experiment. The extracts studied can be classified in category 4 with an LD50 between 300 and 2000mg/kg.

**KEYWORDS:** *Lagenaria siceraria*; acute toxicity; Niger.

## INTRODUCTION

*Lagenaria siceraria* (L siceraria) is a plant of the cucurbitaceae family widely used in traditional medicine. In the Ayurvedic system of medicine, the fruits were used for their cardioprotective, cardiotonic, tonic, diuretic, aphrodisiac, antidote of certain poisons, alternative purgative, cooling effects. They also cure pain, ulcers and fever and is used for chest cough, asthma and other bronchial disorders.[1] The seeds are also edible and considered a good source of vitamin C, β-carotene, vitamin B-complex, pectin and also contain the highest level of choline a lipotropic factor.[2,3,4] Modern phytochemical screening methods have shown the presence of triterpenoid cucurbitacins B, D, G, H,[3,5,6] fucosterol, campesterol.[7] and flavone C-glycosides.[8] L. siceraria seeds are used in headache and migraine-like pain and contain saponins, alkaloids, flavonoids, sterols, fixed essential oils, vitamins.[2,4]

In Niger, the seeds are edible. A study carried out on varieties (*Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus*, *Cucumis prophetarum* and *Lagenaria siceraria*) showed that the species *Lagenaria siceraria* is a potential source of essential amino acids (Haoua et al., 2005; 2007; 2014).[9,10,11] It is also used in traditional medicine to treat or improve prostate disease, intestinal worms, diabetes, cardiovascular disorders and sickle cell disease.[12] Pulps are also used in the treatment of various diseases such as cancer, hemorrhoids, otitis.[13]

According to a report of the Toxicovigilance Coordination Committee, in 2017, several cases of intoxication related to the consumption of certain species of the cucurbit family (*Cucurbita maxima*, *Cucurbita pepo* and *Citrullus colocynthus*) were reported in France. According to this report this toxicity is related to the cucurbitacins present in these species. This study was conducted to assess the level of toxicity of the pulp and seeds of *Lagenaria siceraria* used in traditional Nigerian pharmacopoeia. Specifically, the number of deaths of mice per dose, the influence of the extracts on water and food consumption and body weight were counted.

## MATERIALS AND METHODS

### Plant material

The plant material consists of fruits of Lagenaria siceraria harvested in six (6) regions out of the eight

regions of Niger, namely Niamey, Tahoua, Tillabéry, Zinder, Maradi and Dosso.

**Animal material**

Adult female white mice weighing 20-30g were used. These mice were 8-10 weeks old. They come from the animal house of the Faculty of Science and Technology (Abdou Moumouni University of Niamey). These animals were acclimatized for five (5) days under standard conditions (12 hours of light and 12 hours of darkness at room temperature 25 ± 1°C). During this acclimatization time, these mice had free access to food (mixture of bran, peanut, fish) and tap water.

**Preparation of samples**

**Degreasing**

The previously harvested fruit was crushed and separated from its seeds and pulp. The latter were separately crushed. The powder obtained from the seeds was defatted by percolation of hexane in Soxhlet.

**Decoction**

60g of each powder (seeds and pulp) are introduced into a flask with 600ml of distilled water. The mixture is boiled for 3 hours. After cooling and filtering with filter paper, the decoction is evaporated dry in an oven set at 70°C. The brown lyophilisate is thus obtained. The sample preparation diagram is shown in Figure 1.

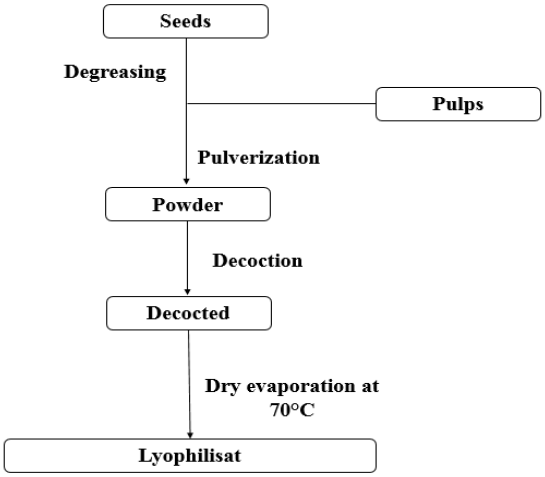


**Figure 1: Sample diagram.**

**Determination of acute toxicity**

OECD Test Guideline No. 423 for Testing of Chemicals, adopted on December 17, 2001, was used to determine the acute toxicity of our samples. According to this guideline, a dose of 300mg/kg (bw) was chosen. Female mice were fasted but allowed to drink tap water for 3 hours prior to administration of the extract. The administration of the extracts is carried out orally.

Pre-weighed mice were divided into thirteen (13) groups of three (3), of which one group served as a control, six (6) received the pulp extract and the remaining six (6) received the seed extract from each region. The control lot received 1ml/100g of distilled water.

After administration of the extract, the mice were observed individually for 30 minutes and then hourly for the first 4 hours. The observations focused on the general behaviour of the mice (coat, tremor, grooming, diarrhoea, motility, stool appearance). The number of deaths per batch was assessed during the first 24 hours after administration of the sample. In case there is 0 or 1 death in the batch of treated mice, another batch of 3 mice receives the same dose of the aqueous extract at 300mg/kg. This test lasted 14 days. During this time parameters such as food and water consumption and body weight of the mice were measured twice a week. [14]

**Statistical analysis**

The statistical analysis was performed using origin6 and Minitab software. Results are expressed as mean ± standard mean error (m ± s.e.m.). The single-factor ANOVA statistical test coupled with the Fisher comparison method was used.

**RESULTS**

The acute toxicity test carried out on the various aqueous extracts of seeds and pulp of *Lagenaria siceraria* (Mol) Standl at a dose of 300mg/kg did not cause any deaths in the mice within 24 hours after administration. According to the Globally Harmonized Classification System (GHS) of the OECD, these extracts are classified as category 4 with an LD50 > 300-2000 mg/kg bw.

**Evaluation of the toxicity of *Lagenaria siceraria* seed extracts**

**Evaluation of behavioural parameters**

Table 1 shows the behaviours (grooming, coat, tremor, motility, stool appearance) of mice treated orally with 300mg/kg (bw) of seed and pulp extracts as a function of time. The analysis in Table 1, shows a change in some behaviours in the mice. Indeed, the signs of toxicity observed are immobility and a straightening of the hair of the mice. Two hours after dosing, the mice found their mobility normal and after four hours their coats were also normal.

**Table 1: Effects of extracts of seeds and pulp of Lagenaria siceraria on mice treated with 300mg/kg dose.**

| | Mouse | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Period** | **1h** | **2h** | **3h** | **4h** | **J3** | **J7** | **J9** | **J13** |
| **grooming** | Ab | Ab | N | N | N | N | N | N |
| **Coat** | Ab | Ab | Ab | Ab | N | N | N | N |
| **tremor** | N | N | N | N | N | N | N | N |
| **motility** | Ab | Ab | N | N | N | N | N | N |
| **Stool appearance** | N | N | N | N | N | N | N | N |
| **Number of dead** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

N : Normal; Ab: abnormal

**Effects of aqueous seed extracts on water consumption**

The variation in the amount of water consumed in the mice receiving the different seed extracts and the control mice receiving distilled water is shown in Figure 2. The analysis of this figure shows a significant decrease ($p<0.01$) in the volume of water consumed by the mice compared to the control lot, for all days of the measurements, 3rd, 7th, 9th and 13th days (J3, J7, J9 and J13), except the 7th day for the seed extracts from Dosso (Figure 2a), Niamey (Figure 2c) and Tahoua (Figure 2d). On the other hand, we note a significant increase on D7 for Maradi (Figure 2b) and Tahoua (Figure 2d) seed extracts. An increase in water consumption is observed for all days for Tillabery (Figure 2e) and Zinder (Figure 2f) seed extracts.

**Effects of aqueous seed extracts on food consumption**

A significant decrease in food consumption was observed on days J3, J7 and J13 in mice treated with Dosso (Figure 3a), Niamey (Figure 3c) and Tahoua (Figure 3d) seed extracts. Maradi (Figure 3b), Tillabéry (Figure 3e) and Zinder (Figure 3f) seed extracts caused a significant increase in food consumption in the treated mice.

**Effects of aqueous seed extracts on weight gain**

Aqueous seed extracts have an influence on the weight gain of treated mice. A significant increase ($p<0.01$) in weight gain was observed in mice treated with Maradi (Figure 4b), Tillabery (Figure 4e) and Zinder (Figure 4f) seed extracts.

**Evaluation of the toxicity of Lagenaria siceraria pulp extracts**

**Effect of pulp extracts on water consumption**

For the mice that received the aqueous extracts from the pulps, a significant increase in water intake was observed daily for Tillabery (Figure 5e) and Zinder (Figure 5f) extracts. For pulp extracts from other regions, an increase was only observed on days 3 and 7 (Figure 5a, 5b, 5c and 5d).

**Effects of Pulp Extracts on Food Consumption**

Regarding food consumption, pulp extracts caused a statistically significant decrease on the 3rd day for aqueous extracts of Dosso (Figure 6a), Niamey (Figure 6c) and Tillabery (Figure 6e). From the 3rd day, for these same extracts, there was a significant increase in food intake. On the other hand, for all days of measurement, Maradi (Figure 6b) and Zinder (Figure 6f) extracts caused an increase in food intake.

**Effects of aqueous pulp extracts on weight gain**

At this level, a decrease in the weight of the treated mice was observed on the 3rd and 13th days with the aqueous extracts of Tahoua (Figure 7d) and Niamey (Figure 7c) pulp. The weight of mice treated with Dosso pulp extracts decreased during the experiment.

The pulp extracts (Maradi, Tillabéry and Zinder) caused an increase in weight weight due to increased food and water consumption. Nevertheless, the pulp extracts (Dosso, Niamey and Tahoua) caused a disturbance of the different parameters studied, i.e. an increase in food consumption followed by a decrease in weight and vice versa.

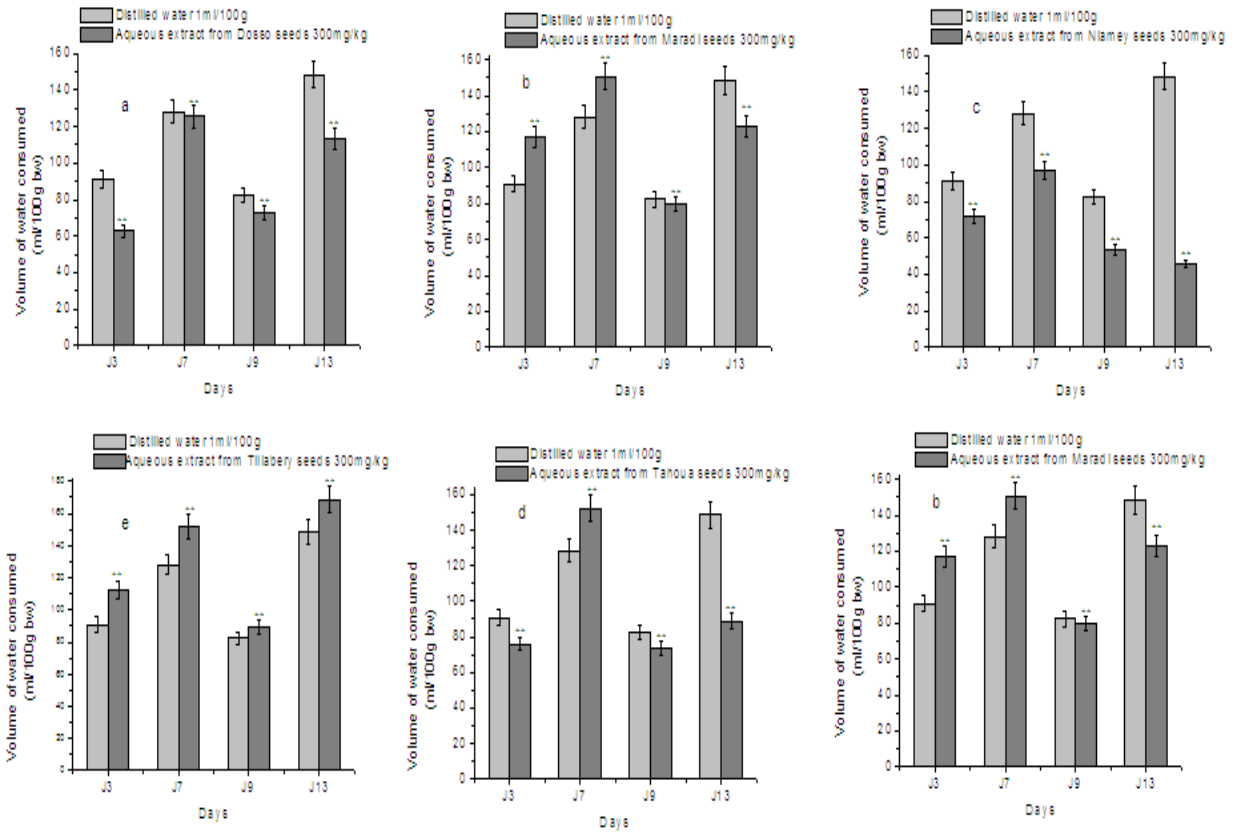


**Figure 2: Effect of the aqueous extract of the seeds of *Lagenaria siceraria* of Dosso (a), Maradi (b), Niamey (c), Tahoua (d), Tillabéry (e) and Zinder (f) on the water intake. Each value represents the mean ± ESM; with ** p <0.01; significant difference compared to the control group distilled water ; n = 3 mice.**

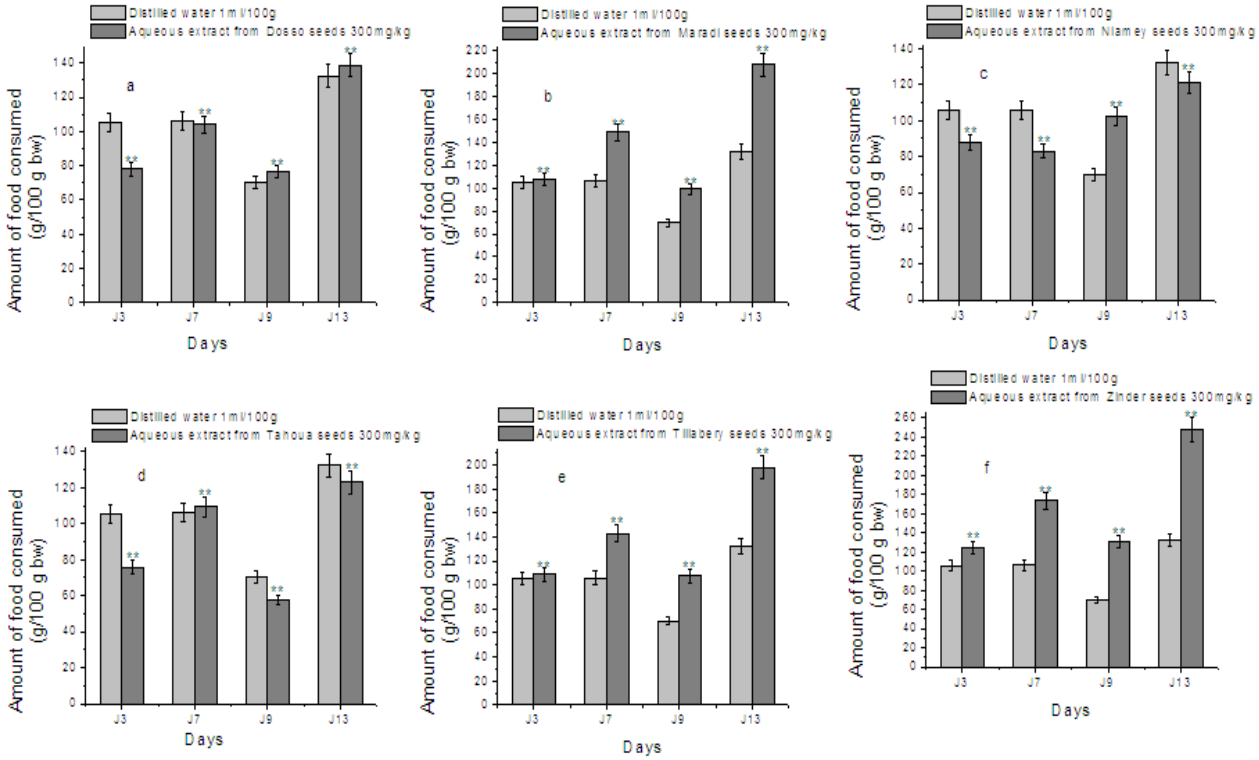


**Figure 3: Effect of the aqueous extract of the seeds of *Lagenaria siceraria* of Dosso (a), Maradi (b), Niamey (c), Tahoua (d), Tillabéry (e) and Zinder (f) on the on food intake. Each value represents the mean ± ESM; with ** p <0.01; significant difference compared to the control group distilled water ; n = 3 mice.**

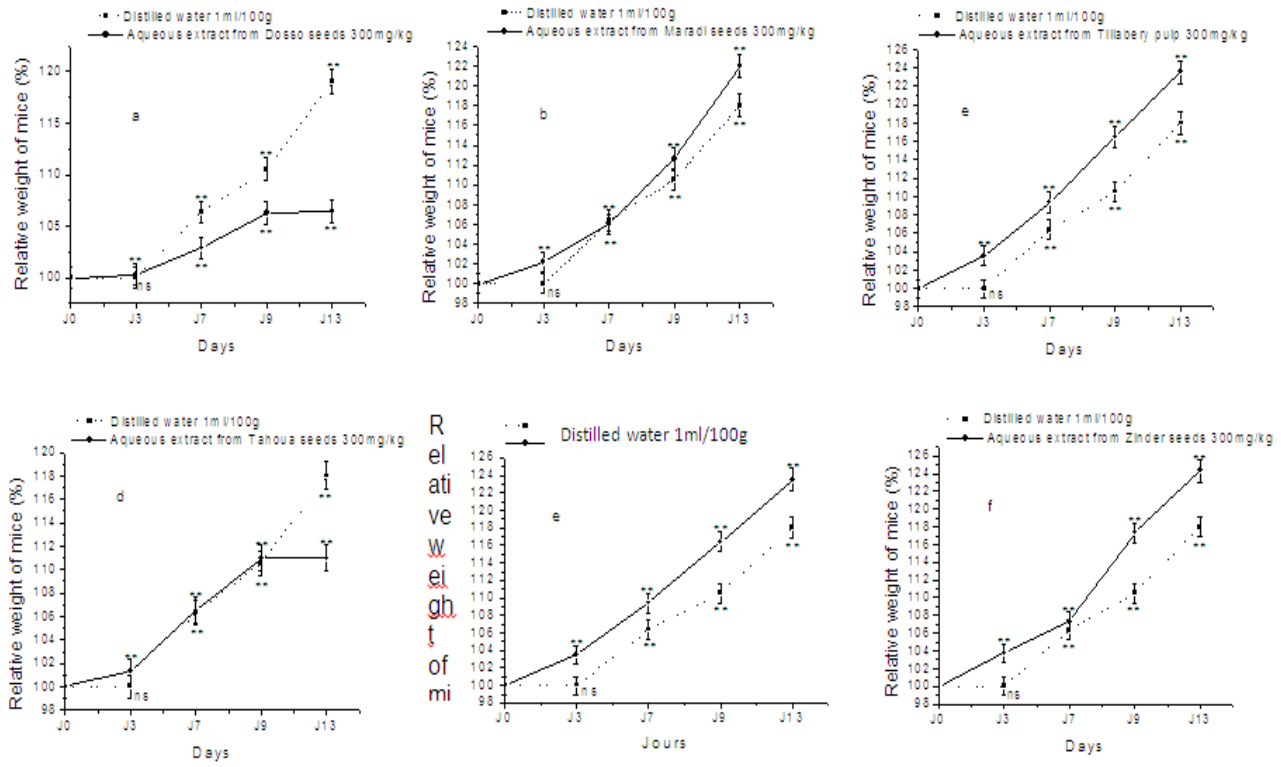


**Figure 4: Effect of the aqueous extract of the seeds of *Lagenaria siceraria* of Dosso (a), Maradi (b), Niamey (c), Tahoua (d), Tillabéry (e) and Zinder (f) on the weight evolution of the mice. Each value represents the mean ± ESM; with ** p <0.01 ; significant difference compared to the control group distilled water ; n = 3 mice.**

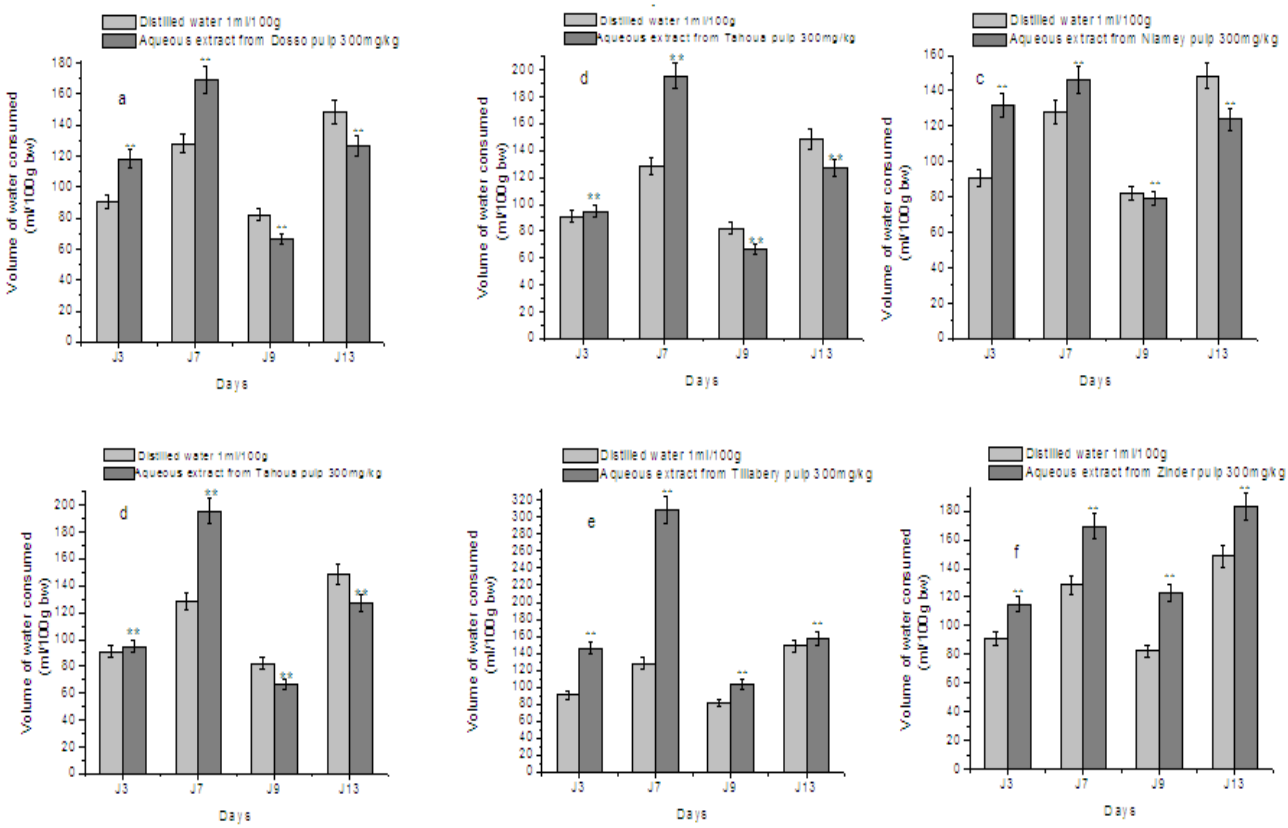


**Figure 5: Effect of the aqueous extract of the pulp of *Lagenaria siceraria* of Dosso (a), Maradi (b), Niamey (c), Tahoua (d), Tillabéry (e) and Zinder (f) on the water intake. Each value represents the mean ± ESM; with ** p <0.01; significant difference compared to the control group distilled water; n = 3 mice.**

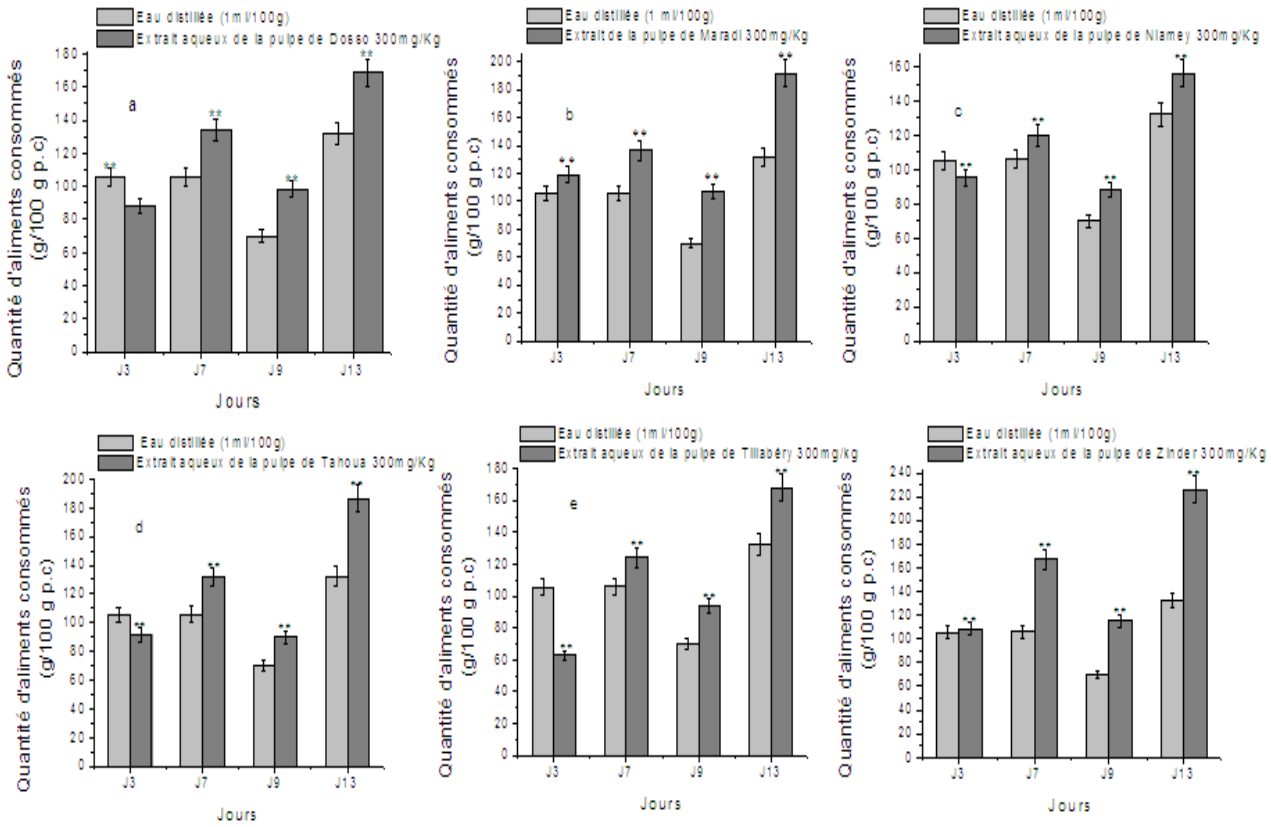


**Figure 6 : Effet de l'extrait aqueux des pulpes de *Lagenaria siceraria* de Dosso (a), de Maradi (b), de Niamey (c), de Tahoua (d), de Tillabéry (e) et de Zinder (f) sur la sur la prise alimentaire. Chaque valeur représente la moyenne ± ESM ; avec **p<0,01 ; différence significative par rapport au lot témoin eau distillée ; n=3 souris.**

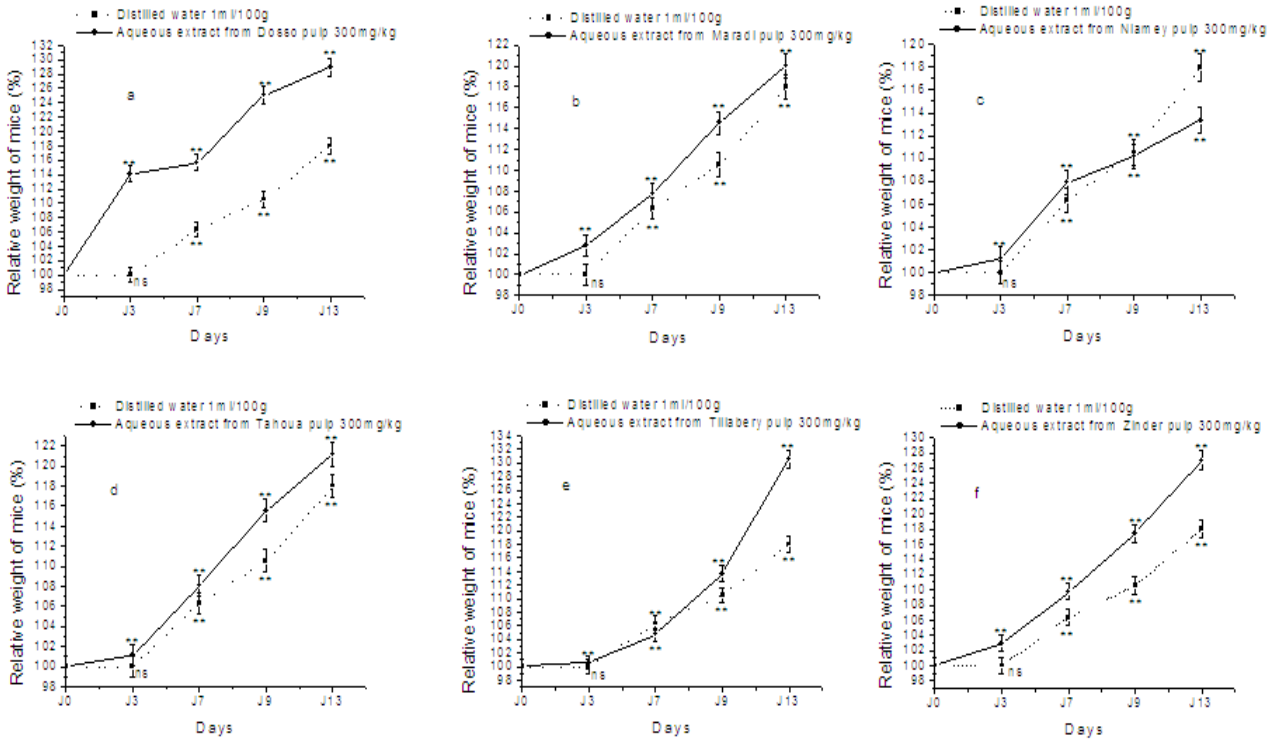


**Figure 7: Effet de l'extrait aqueux des pulpes de *Lagenaria siceraria* de Dosso (a), de Maradi (b), de Niamey (c), de Tahoua (d), de Tillabéry (e) et de Zinder (f) sur l'évolution pondérale des souris. Chaque valeur représente la moyenne ± ESM ; avec **p<0,01; différence significative par rapport au lot témoin eau distillée ; n=3 souris.**

## DISCUSSION

The medicinal use of cucurbits in general and Lagenaria siceraria in particular is widespread in Niger. This species is well known for its nutraceutical use. In order to guarantee the safety of the parts of the plant used in traditional medicine, the present study was devoted to the study of the acute toxicity of the aqueous extracts of the seeds and pulps of Lagenaria siceraria collected in the six (6) regions of Niger. To our knowledge, there is little or no data on the toxicity of these aqueous extracts. Therefore, the results obtained cannot be compared and discussed with previous data.

Oral administration of the aqueous extracts of seeds and pulp of Lagenaria siceraria did not cause any severe change in behaviour. Nevertheless, immobility, straightening and abdominal contractions were observed in treated mice. This could be due to the cucurbitacins contained in these extracts. According to the report of the Toxicovigilance Coordinating Committee, the toxicity of cucurbitacins is mainly related to these cucurbitacins and manifests itself in digestive disorders.[15]

These extracts showed an influence on the quantities of water and food consumed as well as on weight gain. At a dose of 300mg/kg bw, aqueous extracts of Dosso, Maradi, Tillabery and Zinder seeds and pulp caused an increase in the relative weight of mice following consumption of water and food. This increase in body weight could be attributed to high food intake probably due to the administration of these aqueous extracts. A similar observation has been reported in the literature. According to the study, plant extracts stimulate the appetite of treated mice.[16]

For the aqueous extracts of seeds and pulp of Niamey and Tahoua, a disturbance is observed between weight gain and the amount of food consumed. Indeed, an increase in food consumption related to weight loss is observed. According to a study conducted on aqueous extracts of the fruits of Lagenaria siceraria, they quickly help to lose weight because of their high dietary fiber content and their low fat and cholesterol content. [17] According to Keltoum[18] a decrease in animal weight is probably due to the toxic effects of a substance. A study conducted on the fruit of *Citrullus colocynthis* showed that a decrease in animal weight is related to a decrease in organ weight. [19] This decrease in organ weight could be due to the influence of these extracts.

The effects of pulp or seed extracts vary by region. This difference in observed effects could be explained by the influence of several factors. Indeed, according to Sofowora,[20] the composition of a plant in secondary metabolites varies according to the geographical location, the organ removed, the period, the time of removal and storage conditions. According to this author, secondary metabolites are responsible

## CONCLUSION

This study showed that the administration of aqueous extracts of the seeds and pulps of Lagenaria siceraria did not cause any deaths in the treated mice. Therefore, at a dose of 300mg/kg (bw) the extracts are non-toxic. In addition, some extracts such as those of Tillabery and Zinder seeds and Maradi, Tillabery and Zinder pulps caused at 300mg/kg (bw) a weight gain in the mice following an increase in food consumption. Nevertheless, within two hours after administration of all extracts, changes in the general behaviour of the mice were observed. The LD50 of these extracts ranged from 300 to 2000mg/kg bw. Further studies are required to better understand the mechanisms involved in the various effects observed.

## CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

## CONTRIBUTIONS OF THE AUTHORS

All authors contributed to this work. They have also read and approved this manuscript.

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