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Subacute toxicity assessment in rats of aqueous extract from *Abrus precatorius* Leaves (Fabaceae) a medicinal plant used in the treatment of diabetes

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Abstract
Abrus precatorius is recognized in traditional medicine for its therapeutic virtues on various diseases such as diabetes. The study consisted of evaluating the subacute toxicity in rats of the aqueous extract of the leaves of *A. precatorius*. The aqueous extract obtained by the traditional method (Decoction). The subacute toxicity study was carried out for 21 days. The rats (n=12) were distributed into two groups of six. The treated batch receives orally day (Morning / evening) for 7 days at two doses of extract aqueous (ETAAP 40 mg/mL) against the control batch which receives only distilled water. The rats were observed 14 days after stopping treatment and some biochemical parameters related to liver function (Glutamic Pyruvic Transaminase and Glutamic Oxaloacetic Transaminase) and renal function (Creatinine) were sought. The results of subacute toxicity showed that the experimental effective dose of aqueous extract did not cause significant variation of the transaminase and creatinine in normal rats treated against the control group. The study showed no deaths and no clinical signs of toxicity in rats.

Keywords: *Abrus precatorius*, aqueous extract, subacute toxicity, diabetes

Introduction
Abrus precatorius Linn (Fabaceae) is known mainly for its medicinal properties to cure various diseases. The leaves are recognized for their many therapeutic virtues in traditional medicine. The leaves are used as expectorant and antitussive [1], laxatives, against conjunctivitis, eczema, stomach trouble and as an aphrodisiac [2]. They are used to cure asthma and bronchitis [3]. In the department of Agboville (Southern Côte d'Ivoire) Abbey and Krobou peoples use the leaves of *Abrus precatorius* to treat gyneco-obstetric disorders and to make childbirth easier [4]. In the west of Nigeria, the leaves decoction is used in the treatment of diabetes [5]. The screening phytochemical of aqueous extract of *A. precatorius* leaves, revealed the presence of several chemical groups: alkaloids, tannins, flavonoids (flavones), saponins, quinones (Coumarins), sterols, triterpenes and reducing compounds, and it is not toxic by oral route in rats [6, 7]. The studies in diabetes showed that the aqueous extract of *A. precatorius* leaves at effective dose (ETAAP 40 mg / ml) decreases the hyperglycemia caused in rats with good blood sugar regulation [7]. This study was carried out to evaluate the subacute toxicity of the experimental effective dose of extract (ETAAP 40 mg / ml) by determining some seric biochemical parameters related to liver (Glutamic Pyruvic Transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT)) and kidney (creatinine) in order to bring out its atoxic character in normal rats.

Materials and Methods
Plant collection
The leaves of *Abrus precatorius* were collected in an urban area of Abidjan (Southern Côte d'Ivoire) in October, 2014. The plant had already been identified at the National Floristics Center of Abidjan (Côte d'Ivoire) on the issue: *Abrus precatorius* (Fabaceae): Aboudé-Mandéké (Côte d'Ivoire), 23 May 1990 N'Guessan Koffi 165 [4].

Preparation of extracts

The aqueous extract obtained by decoction from a powder of leaves previously dried [8]. 10 g of leaves powder were introduced into a triple-neck round bottom of 250 mL, and then 100 mL of distilled water were added. A round-bottom was topped with a cooler connected to a faucet opened by a pipe. The round-bottom was put down into a warm balloon (ELECTROMANTLE) maintained in a constant temperature of heating for one hour. After cooling, the mixture was filtered with cotton wool three times and the obtained filtrate was put into the stove (SELECTA) at 55 °C for 24 h. The extract was dried and the aqueous extract was obtained. Extraction was repeated several times to obtain a sufficient quantity.

Experimental animals

The experiments were carried out at the animal house of Faculty of Science and Technology, Sultan Moulay Slimane University, Beni-Mellal (Morocco).

The male rats *Rattus norvegicus* strain Wistar weighing between 180-183 g were used for this study. The animals were placed in plastic cages containing chips of wood renewed every 3 days. The rats (n=12) were acclimated to the conditions of laboratory (a temperature of 20 to 22 °C, 12 hours of light and 12 hours of darkness) for 7 days then divided into 2 homogeneous groups of 6. All animals were allowed free access to water and fed with standard pellet raw chaw. The animals were fasted for 18 hours before the administration of the gavage extract against the control group, they were deprived of food but not of water.

Subacute toxicity of aqueous extract of *A. precatorius* leaves in rats

The toxicology study was adapted to the method used by Lébri *et al*., [6]. During this 21-day study, the 12 animals were distributed into two groups of six. They were gavaged each day at two doses of the test substances (Morning / evening) for 7 days against control distilled water. The animals were observed 14 days after stopping treatment and some biochemical parameters related to hepatic and renal profile were sought.

Determination of biochemical parameters in relation to the liver and kidneys

The determination of serum parameters in relation to liver function (Transaminases TGO/TGP) and renal function (Creatinine) was performed. Blood samples were taken in 4 ml red cap tubes (VACUETTE) from the caudal extremity of the rats, before treatment, one week after treatment and two weeks after treatment discontinuation. The collected blood was centrifuged at 3,000 rpm for 10 minutes. The serum was collected in Eppendorff tubes and stored at -20° C for serum parameter analyzes. Serum biochemical parameters were analyzed, using commercially available standard dosing kits (SPINREACT, Spain), by enzymatic kinetics methods (TGP: Glutamic-Pyruvic Transaminase or ALT: Alanine transaminase, GOT: Glutamic oxaloacetic transaminase or AST) and by Jaffé's method in chemical kinetics (Creatinine). These parameters are determined by a UV-Visible double-beam spectrophotometer (SPECORD® 200 PLUS, Analytik Jena) controlled by a computer system and managed by an application software.

Observation of animals

During the 21-day study, clinical signs of possible toxicities and death were sought during (7 days) and after treatment (14 days) of the animals at the effective dose extract against the control. The signs of toxicity sought were: apathy, excitement, breathing disorders, refusal of food, oral bleeding, nasal bleeding, abdominal pain (Contortion), coma, diarrhea, tremor, convulsion and other signs.

Statistical analysis

The results are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) with repeated measures was employed to compare the results according to the administered doses and times of treatment. Analysis of variance was considered significant when the level of probability (p) was < 0.05; if $p<0.01$, this difference is considered as very significant; if highly significant $p<0.001$.

Results

Evolution of transaminases (GOT and GPT)

The evolution of transaminases was followed by groups and by comparison between groups.

Glutamic oxaloacetic transaminase (GOT)

The histogram shows a non-significant increase ($p> 0.05$) of glutamic oxaloacetic transaminase (GOT) after one week of gavage (D7). GOT increased from 206.0±28.62 to 216.6±56.46 IU / L, an increase of 5.14% on the first day (D0). Two weeks after cessation of gavage (D21) a non-significant decrease in transaminase is recorded, it increases to 107.2±29.36 IU / L, a decrease of 47.96%. The results show a non-significant increase in transaminase (GOT) after one week of gavage and a not significant decrease two weeks after gavage was stopped for 21 days (Figure 1). A not significant increase ($p>0.05$) of transaminase (GOT) is recorded after one week of gavage (J7). It went from 191.4±32.6 to 210.0±42.98 IU / L, an increase of 9.71%. Two weeks after cessation of force-feeding, an insignificant increase is recorded by report on the first day (D0). Transaminase increased to 203.2±22.05 IU / L, an increase of 6.16%. These results show that, in rats fed to the extract (ETAAP 40 mg / ml), the transaminase does not significantly increase after one week of gavage and two weeks after cessation of gavage during 21 days (Figure 2). The results in Figure 1 and 2 show that the transaminase increases insignificantly ($p> 0.05$) after one week of gavage in both groups. Two weeks after gavage, GOT increased insignificantly in the extract-treated rats (ETAAP 40 mg / ml) whereas transaminase decreased insignificantly in the control rats. However, Figure 3 shows a not significant difference ($p> 0.05$) in the change in GOT between the two groups after one week of gavage (D7). Two weeks after cessation of force-feeding (D21), an insignificant difference ($p<0.05$) was recorded between the two groups.

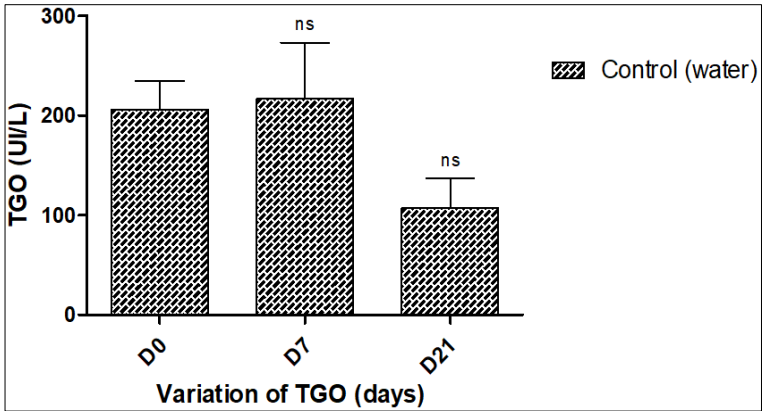
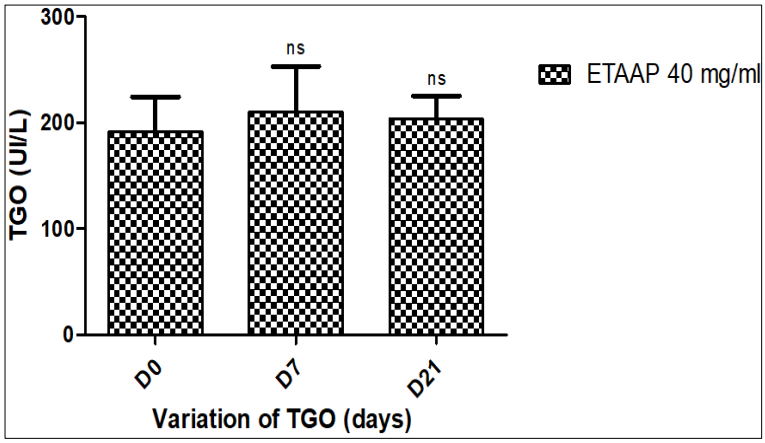
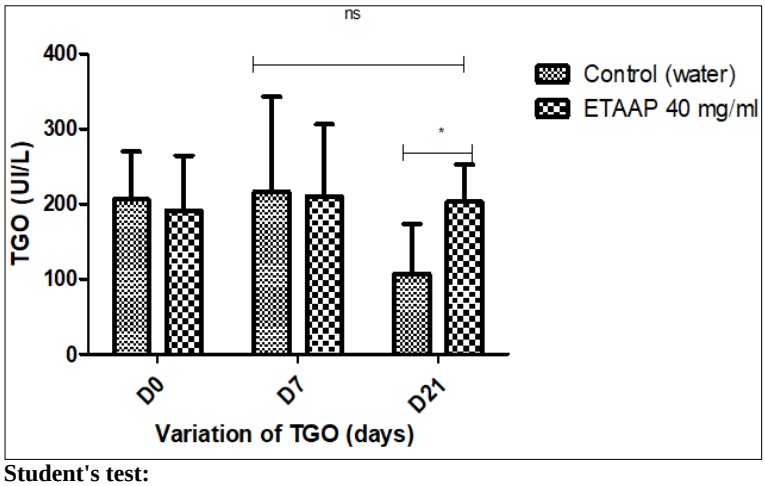


Fig 1: Variation of GOT for 21 days in normal rats gavaged for one week at two doses of the control (water)



Dunnett's test
ns p> 0,05: not significant versus on D0 (n = 6)

Fig 2: Variation of TGO for 21 days in normal rats gavaged for one week at two doses of the extract (ETAAP 40 mg/ml)



Student's test:
ns p> 0.05: not significant versus on D0 (n = 5)
*p< 0.05: significant versus on D0

Fig 3: Variation of GOT for 21 days in normal rats treated for one week at two doses of the extract (ETAAP 40 mg/ml) against the control (water)

Glutamic-Pyruvic Transaminase (GPT)

The histogram shows an insignificant increase (p<0.05) of Glutamic-Pyruvic Transaminase (TGP) after one week of gavage (D7), from 68.00±17.97 to 101.2±25.16 IU. / L, an increase of 48.82%. Two weeks after cessation of force-feeding (D21), an insignificant increase (p> 0.05) in the order of 16.76% is recorded with a value of 79.40±18.23 IU / L.

These results show that transaminase (GPT) increases significantly after one week of gavage and two weeks after gavage the GPT increases insignificantly (Figure 4).

An insignificant increase (p>0.05) in GPT was recorded after one week of gavage of rats at the extract (ETAAP 40 mg / ml), from 73.80±14.92 to 92±43.94 IU / L, an increase of 19.78%. Two weeks after cessation of gavage (D21), the transaminase decreased insignificantly (p> 0.05) compared

to the first day (day 0) without force-feeding. It increases to 66.40±12.60 IU / L, a decrease of 10.02%. These results show that in rats fed with ETAAP 40 mg / ml, the transaminase (GPT) increases and decreases insignificantly respectively after one week of gavage and two weeks after cessation of gavage (Figure 5).

Figures 4 and 5 show that GPT increases insignificantly (p> 0.05) after one week of gavage in the treated group while the increase is significant (p<0.05) in control rats in relation to the first day (D0). Two weeks after gavage, TGP decreased non-significantly (p> 0.05) in treated rats while in control rats a non-significant increase was recorded. However, transaminase (GPT) varies insignificantly (p> 0.05) between the two groups (Figure 6).

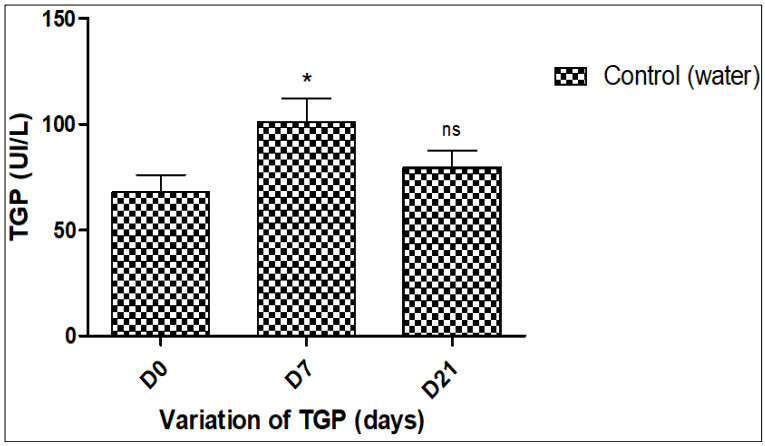
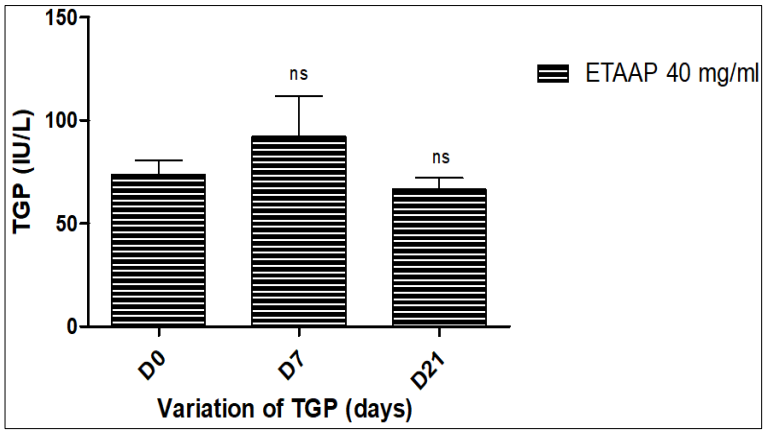
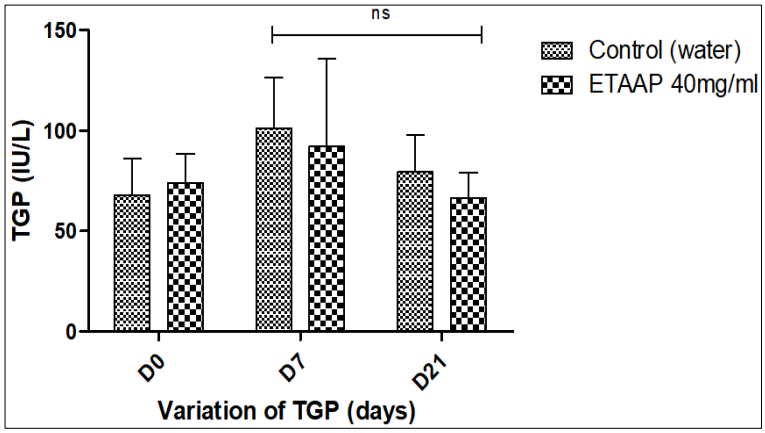


Fig 4: Variation of GPT 21 days in normal rats gavaged for one week at two doses of the extract (ETAAP 40 mg/ml) against the control (water)



Dunnett's test
ns p> 0,05: not significant versus on D0 (n = 6)
*p< 0, 05: significant versus on D0 (n = 6)

Fig 5: Variation of TGP 21 days in normal rats treated for one week at two doses of the extract (ETAAP 40 mg/ml)



Student's test:
ns p> 0.05: not significant versus on D0 (n = 6)

Fig 6: Variation of TGP for 21 days in normal rats treated for one week at two doses of the aqueous extract of *Abrus precatorius* leaves (ETAAP 40 mg / ml) against the control (water)

Evolution of creatinine: In the control rats, after one week of gavage, the creatine increases insignificantly (p> 0.05), it goes from 9.5±2.19 to 13.17±3.5 mg / l, an increase of 38.66%. Two weeks after the cessation of force-feeding, an insignificant decrease (p> 0.05) is recorded, the transaminase passes to 8.4±2.25 mg / l with a decrease of 11.70% compared to the first day (D0).

These results show a significant increase after one week of gavage and two weeks after gavage, creatinine does not decrease significantly (Figure 7). In treated rats, after one week of gavage, a non-significant increase (p> 0.05) in creatinine was recorded, from 9.5±2.21 to 12.73±5.8 mg / l, an increase of 34.11%. Two weeks after the end of gavage, creatinine decreases insignificantly. Creatinine increased to 7.3±4.08 mg / l, a decrease of 22.57% compared to the first day (D0). These results show a non-significant increase in creatinine after one week of gavage and two weeks after gavage creatinine decreases in a non-significant manner (Figure 8).

Figures 7 and 8 show a non-significant increase (p> 0.05) of creatinine in both groups after one week of gavage. Two weeks after force-feeding, creatine decreased not significantly in both groups. However, creatinine varies non-significantly (p> 0.05) between the two groups within 21 days (Figure 9).

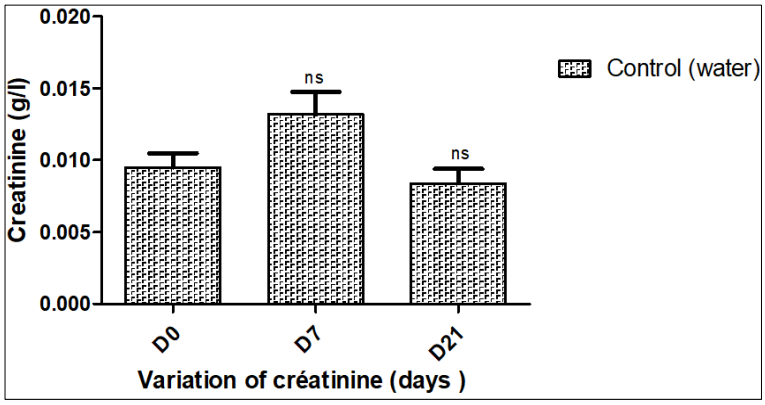
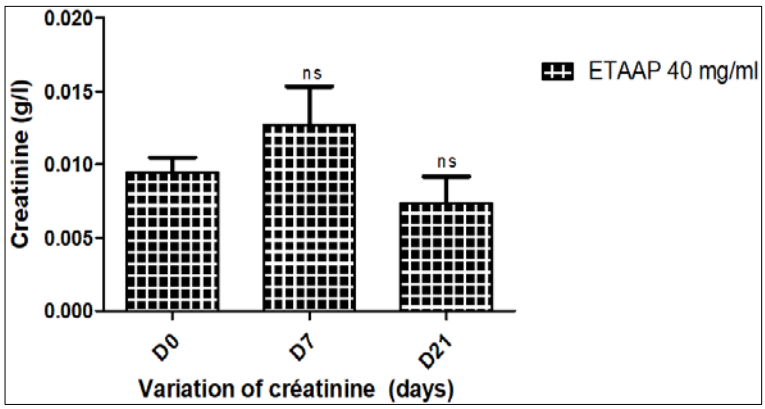
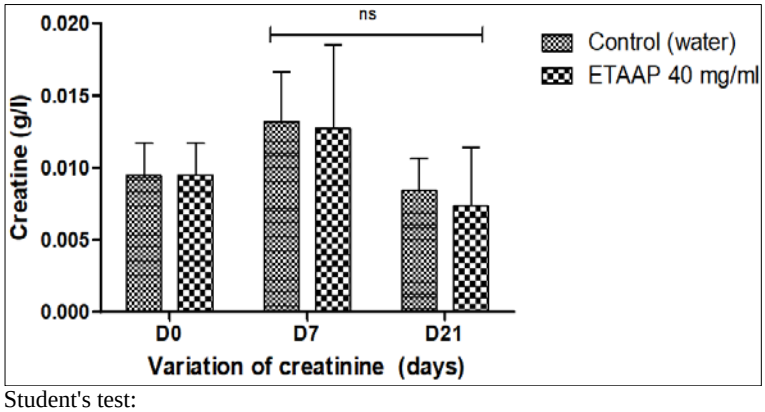


Fig 7: Variation of creatinine for 21 days in normal rats treated for one week at two doses of the control (Water)



Dunnett's Test
ns p> 0,05: not significant versus on D0 (n = 6)

Fig 8: Variation of creatinine for 21 days in normal rats treated for one week at two doses of the aqueous extract of *Abrus precatorius* leaves (ETAAP 40 mg / ml)



Student's test:
ns p> 0.05: not significant versus on D0 (n = 5)

Fig 9: Variation of creatinine for 21 days in normal rats treated for one week at two doses of the aqueous extract of *Abrus precatorius leaves* (ETAAP 40 mg / ml) against the control (water)

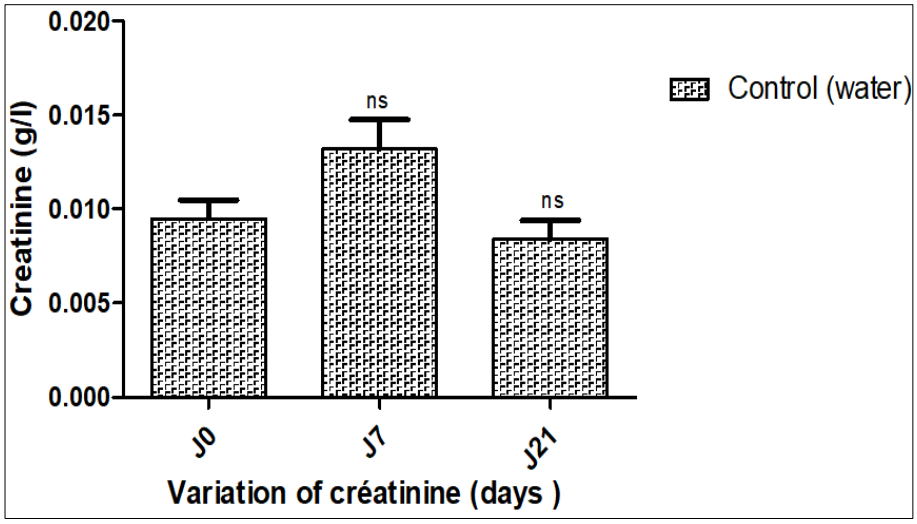
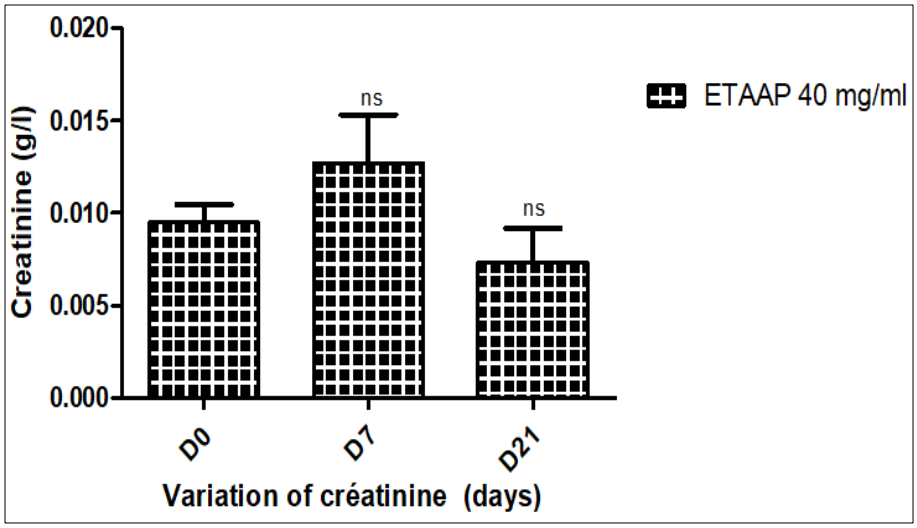
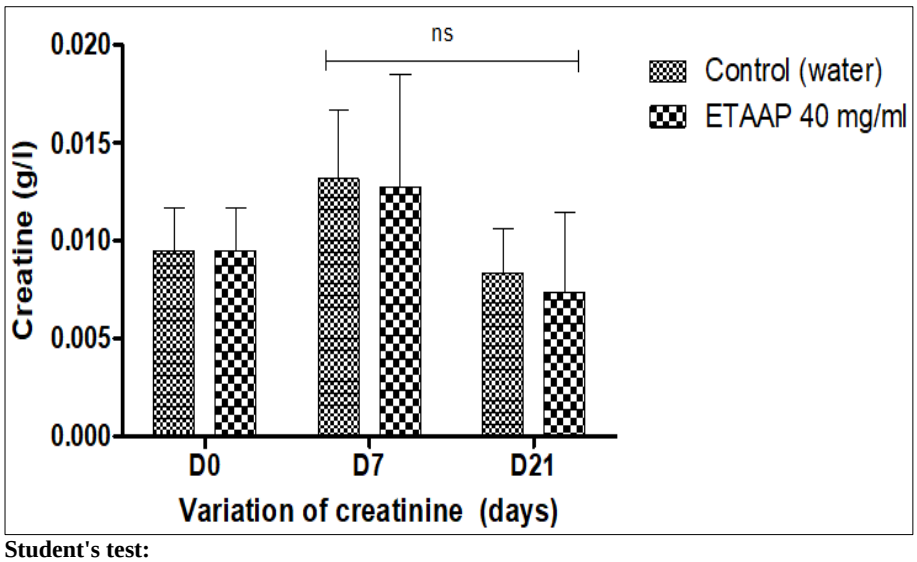


Fig 10: Variation in creatinine during the 21 days in normal rats subjected to daily gavage for one week at two doses against the control/vehicle (Water)



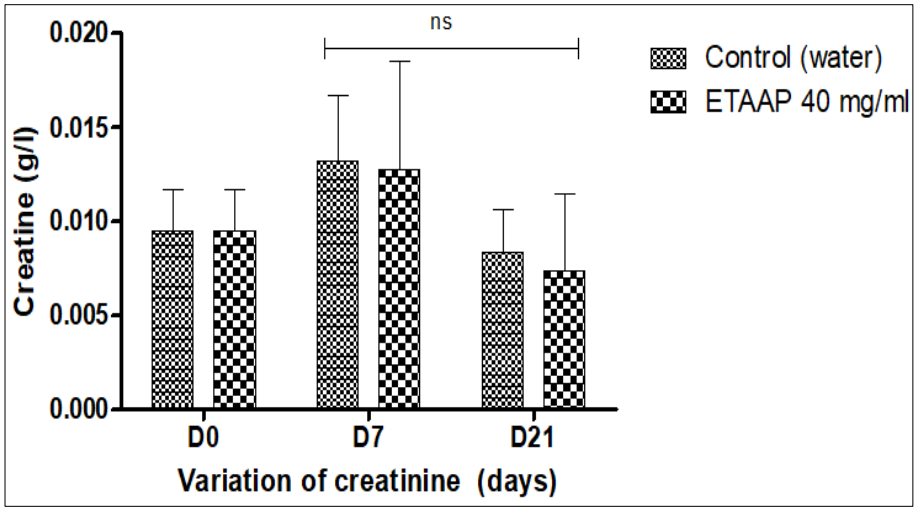
Dunnett's test
ns $p > 0.05$: not significant versus on D0 (n = 6)

Fig 11: Variation in creatinine for 21 days in normal rats treated for one week at two doses of the aqueous extract of *Abrus precatorius* leaves (ETAAP 40 mg / ml)



Student's test:
ns $p > 0.05$: not significant versus on D0 (n = 6)

Fig 12: Variation of creatinine for 21 days in normal rats treated for one week at two doses of the aqueous extract of *Abrus precatorius* leaves (ETAAP 40 mg / ml) against the control (Water)



Student's test
ns $p > 0.05$: not significant versus on D0 (n = 6)

Fig 13: Variation in creatinine over 21 days in normal rats treated for one week at two doses of the extract (ETAAP 40 mg / ml) against the control (water)

Results of animals observation

The subacute oral toxicity study showed no deaths and no clinical signs of toxicity (Table 1) following two doses administration of the aqueous extract of the effective dose of *Abrus precatorius* leaves (ETAAP, 40). mg / ml) for 7 days.

Table 1: Results of animals observation for 21 days

Signs clinics of toxicity	Batches control (Distilled water)	Batches treated (ETAAP 40 mg/ml)
Apathy	-	-
Excitation	-	-
Breathing Disorders	-	-
Refusal of food	-	-
Oral bleeding	-	-
Nasal bleeding	-	-
Abdominal pain (contortion)	-	-
Coma	-	-
Diarrhea	-	-
tremor	-	-
Convulsion	-	-
Mortality	-	-

Sign "-" indicates the absence of clinical signs related to animal behavior.

Discussion

The leaves and seeds of *Abrus precatorius* continent abrin [2] which is a very toxic compound [9]. However, the study of the acute oral toxicity in rats of the aqueous extract of the leaves showed no death and no sign of visible toxicity despite this rich natural chemical group [4]. Among these compounds, alkaloids, sterols and triterpenes may be responsible for the antidiabetic activity of the plant [10]. The aqueous extract of *Abrus precatorius* leaves possesses potential antidiabetic effect in rats with a good regulation of glycemia at the experimental effective dose (ETAAP 40 mg / ml) [7]. The toxicity study consisted in force-feeding the animals twice a day for one week at the effective dose of the extract (ETAAP, 40 mg/ml). The results showed insignificant changes in some biochemical parameters which were recorded between the treated and control rats during the 21-day period. The absence of any significant change in transaminase and creatinine activity indicates that the active ingredients contained in the extract at the effective dose did not cause liver and kidney damage. These results are in agreement with those obtained by Ikechukwu *et al*., [11] whose work has shown that the methanolic extract of *Abrus precatorius* leaves have the capacity to improving liver functions and liver regeneration. The results obtained are close to those of Azzi, [12] whose work showed that total alkaloid extracts (150 and 300 mg / kg bw) or ethanolic cucurbitacin glycosides (75 mg / kg bw) of colocynth seeds after intraperitoneal injection does not cause liver and kidney damage. They are also close to those obtained by Koné *et al*., [13] whose work revealed that the aqueous extract of *Sacoglottis gabonensis* bark (Baille) Urban (Humiriaceae) at any doses does not cause a significant variation in transaminases (GOT, GPT), creatinine, glucose and body weight in rodents. In contrast, the work of Sanogo *et al*., [14], at doses of 300 mg / kg and 1500 mg / kg of the aqueous extract of *Argemone mexicana* caused congestive hepatitis and subacute congestive hepatitis, respectively. The study of toxicity of the aqueous extract of *Abrus precatorius* leaves at the effective dose does not cause a significant change in the parameters evaluated and showed no deaths and no clinical signs of toxicity.

Conclusion

The results of the subacute toxicity show that the aqueous extract of the leaves of *Abrus precatorius* at the effective dose (ETAAP 40 mg / m) does not cause abnormalities on the liver and the kidneys in normal rats. No deaths, no clinical signs of toxicity was observed in rats. This could

confirm the safety of the aqueous extract of *A precatorius* leaves observed in humans in the traditional treatment of several diseases like diabetes. However, it would be necessary to carry out further toxicological studies on the leaves of *A. precatorius* to verify its atoxic character.

Acknowledgments
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