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IN-VITRO AND IN-VIVO TOXICOLOGICAL EVALUATION OF AQUEOUS EXTRACTS FROM BENINESE PLANTS: *SENNA SIAMEA* LEAVES AND *SARCOCEPHALUS LATIFOLUS* STEM ROOTS

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ABSTRACT: The toxicity of aqueous extracts of *S. siamea* leaves, *S. latifolius* roots collected in Benin and their mixture (1/1, w/w), was evaluated on female non-pregnant rats, Albino wistar genus. They were treated for two weeks, with various extracts administered by the oral way at 2000 mg/kg body weight. The biochemical parameters were determinate, histological analysis of the rats was done, and the urinary concentration of neutral gelatinase-associated lipocalin (NGAL) was measured. The treatment of the rats with different extracts not caused undesirable effects and deaths during the fourteen days of observation. Significant changes are relative of a histological aspect of hepatocyte and renal cortex as well as significant increases in the activity of enzyme serum alanine aminotransferases (ALT) and aspartate aminotransferases (AST). The aqueous extracts at 2000 mg/kg were disrupted some biochemical parameters. These changes were confirmed by the rate raised from NGAL noted in the urine of the treated rats.

INTRODUCTION: The plants are harvested and fluently used for foods and medicines purposes ^{1,2}. Alcoholic extracts of leaves of *Senna siamea* have been shown anti-cancerous and antioxidant activities ³.

In West African countries, its peel and its eraser are used to treat cancers and the tumors of the ear, eyes and genital organs. In Benin, the decoction of the root is used against fever, constipation, hypertension and insomnia ⁴. In the north of Nigeria, the peel of *Sarcocephalus latifolius* is used in brewing as a diuretic and anti-helminthic ⁵. Particularly, its root is used to fight against various infections such as malaria and stomach pains ⁶. Other studies used the extracts of peel stems to treat pain, inflammation and malaria effects ^{7,8}. Unfortunately, very little studies have done on the



acute toxicity of those plants which are recognized to have enormous pharmacological wealth. The objective of the present work is to evaluate the toxicity of aqueous extracts of *S. siamea* leaves, of *S. latifolius* roots and their mixture (w/w) on the rats.

MATERIAL AND METHODS:

Material: The leaves of *S. siamea* and the roots of *S. latifolius* were collected in March 2013, respectively in Adjarra and Porto-Novo at Benin. The plants were authenticated at the National herbarium from Abomey-Calavi University. The voucher specimens were kept under the numbers: AA6414/HNB (*S. siamea*) and AA6415/HNB (*S. latifolius*). They were dried in the laboratory room at constant temperature (air conditioning) and carefully powdered with an electric grinder (of Flour MILLS NIGERIA, El MOTOR No 1827). Eggs of Shrimp larvae (*Artemia salina* Leach) commercialized by German company JBL GmbH & Co. KG was used for assessing the cytotoxicity. Male and female albino Wistar rats weighing 150-200 g at the age of months were used for this study. Animals were housed in polypropylene cages and maintained under standard conditions with an alternated cycle of 12 h light and 12 h dark with free access to food and water. The room temperature was maintained at 25 °C with a relative humidity of 35-60%.

Methods:

Extraction and Cytotoxicity Assay: 50 g of each powdered plant were macerated with 500 ml of distilled water for 72 h and filtered three times successively. Then the filtrate was evaporated to dryness at 40 °C using a rotary evaporator (Heidolph efficient Laborota 4000) coupled to a water chiller (Julabo FL 300) to give the crude aqueous extracts⁹.

The cytotoxicity test was performed on *Artemia salina* Leach by the method used by Hounghèmè et al.⁹ The eggs of *Artemia salina* were incubated in seawater until hatching of young larvae (48 h). Then, a series of solutions of each tested crude extract at varying and progressive concentrations were prepared. A defined number (16) of larvae were introduced into each solution. All solutions and control solution containing no active substance was left under stirring for 24 h. Counting under a

microscope the number of dead larvae in each solution was used to evaluate the toxicity of the solution. In the case, where there was death in the control medium, the data was corrected by Abbott's formula¹⁰:

$$\% \text{ death} = 100 \times [(\text{test} - \text{control}) / \text{control}]$$

Data (dose-response) are transformed by logarithm, and the LC₅₀ was determined by linear regression¹¹. Tests were carried out in duplicate.

Acute Toxicity Assay: This test was carried out for aqueous extract of *S. siamea* leaves, *S. latifolius* roots and their mixture on Wistar rats according to Organization for Economic Cooperation and Development (OECD) guidelines¹². Wistar rats (3 males + 3 females) weighing 150-200 g were used for the test. Animals were kept out on fasting overnight before aqueous extract of *Momordica charantia* administration.

After the period of fasting, they were weighed, and plant extract was administered by gavage at the dose of 2000 mg/kg. Following administration of extract, food was held for further 3-4 h followed by observation after 30 min, 1 h, 2 h, 3 h, 4 h, 24 h and once a day till the fourteenth day. Rats were examined for various observations like weight change, tremors, convulsion, salivation, diarrhea, lethargy, sleep, coma, and death. Along with that, the cage side observations like changes in the skin, fur, eyes and behavioral pattern were studied.

Sub-acute Toxicity Assay: The rats were unfortunately distributed in a lot of five animals. 3 lots received 2000 mg/kg respectively of the aqueous extract of leaves of *C. siamea*, of *S. latifolius* roots and their mixture. The animals were treated for 14 days, and the observation continued after two weeks following the treatment. The dose of the daily one hold managed extract was 2000 mg/kg. During the 4 weeks of the survey, the clinic's signs and the toxicity were observed in all animals daily. The blood withdrawal was done since the first day (J₀). It was continued to the 3rd, 7th, 14th and 28th days after administration of the extract. At 7th day, a rat was sacrificed in every group and was dissected; the withdrawal of the kidneys and liver were done for the histological survey.

Determination of Biochemical Parameters: The blood and serum samples were collected by cervical decapitation from anesthetized rats into heparinized bottles for hematological studies. Blood samples collected into non-heparinised bottles were allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Hematological Measurements Blood samples were collected from retro-orbital of the experimental rats in capillary tubes coated with ethylene diamine tetra-acetic acid (EDTA). The tubes were immediately capped, kept at -4 °C and were immediately analyzed for blood parameters using automated Coagulating Sysmex apparatus Type 8999. The parameters included Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using a photoelectric colorimeter (Gallenkamp® and Sons Ltd.; England)^{13, 14}. Serum urea and creatinine levels were determined using the same photoelectric colorimeter¹²⁻¹⁴.

Histological Analysis of Rats Treated at 2000 mg/kg with Extracts: The kidneys and the liver were conserved in formalin (10%) during one week, and then they were cutting in small pieces. These samples are dehydrated in three successive ethanol baths for 30 min (70-75°; 90-95° and 100°) before solved in toluene baths, during 20 min. Then, they were put in two baths of paraffin for every 2 h. The operation was automated with an automaton (TISSUE-TEK® II°) device. The definitive inclusion was then achieved in metallic molds (Shandon [OH] Histocentre). The gotten blocks of kinds of paraffin were cut by a microtome (LEICA RM 2145). The cuts of 5µm of thickness were spread on blades and were dried for 1 h at 37 °C, dehydrated, colored to the hematoxylin-eosin (SHANDON [OH]).

Urinary Concentration Determination of Neutral Gelatinized Associated Lipocalin (NGAL) of Treated Rats: It is about to cram the animals at 2000 mg/kg. Urine was collected at 0, 2, 4, 6, 8, 10, 12, 24 h after stuffing. The NGAL test urinary ELISAT is an immunological micro particular determination by reading at a wavelength of 450 nm for its quantitative determination in urine. It was used to detect sharp renal lesions.

Statistical Analysis: All the data obtained were submitted to statistical analysis using Minitab software Version 1.0. The results were expressed as mean. Once the variance analysis (ANOVA) was performed, the differences were pointed out using the Kruskal-Wallis test. Results were considered statistically significant for $p < 0.05$. Correlations between different values were expressed as a graph using Graph Pad PRISM software version 5.

RESULTS:

Cytotoxicity Activity: The Fig. 1 show the sensibility of extracts of *S. siamea* leaves, *S. latifolius* roots, and their mixture. So, the LC₅₀ values are respectively 0.78 mg/ml, 1.56 mg/ml and of 3.16 mg/ml. The statistical analysis of these results, done by the Student test in non matched sets, permitted us to observe a meaningful difference ($p < 0.05$) between the variables of every extract.

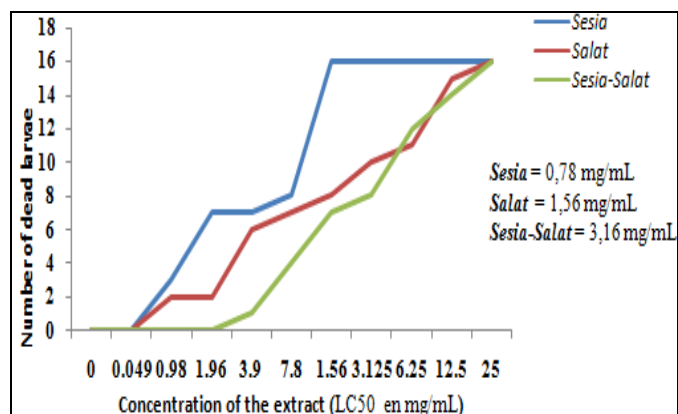


FIG. 1: COMPARATIVE GRAPHS OF THE TOXICITY OF THE EXTRACTS ON THE SHRIMP LARVAE. Sesia: *S. siamea*, Salat: *S. latifolius*; Sesia-Salat: the mixture of *S. siamea* leaves and *S. latifolius* roots (1/1)

The aqueous extract of the mixture decorated less poisonous on the larvae that one of *S. latifolius* roots and *S. siamea* leaves. According to the correspondence table of Mousseux¹⁵, LC₅₀ values proved that the extracts were not toxic. There is a good correlation between toxicity against shrimp larvae and cytotoxicity on 9KB and 9PS (nasopharygien human carcinoma) cells, on the one hand, and cytotoxicity on A-549 carcinoma lung cells and HT -29 colon carcinoma cells, on another hand⁹. So the aqueous extracts and their mixture are not cytotoxic.

Acute Toxicity: No overt undesirable effect and no deaths were recorded during the 14 days of

observation for regular clinic of sequential doses, 300 mg/kg and 2000 mg/kg of body weight of the animal treated with aqueous extracts of *S. siamea* leaves, *S. latifolius* roots and their mixture (v/v). At the time of the experimentation progress, the absence of undesirable effect to the sequential dose of 2000 mg/kg of the body weight of the animal entailed the end of the test.

Evolution of Transaminases on the Treated Rats: The Fig. 2 reveals an increase of the AST; 22.27 times 24 hr after within the rats treated to the aqueous extract of *Salat* to the dose 2000 mg/kg. This increase remains obstinate and reaches 460.6 ± 11.2 UI/L on the 7th day is 30.71 times the value of J₀. One week after (J₁₄), this value regressed and reached 300 ± 10.2 UI/L. Two weeks after the stop of the treatment (J₂₈), the regression persisted and reached a value of 110.2 ± 10.2 UI/L is 7.35 times the value in J₀. To the level of the rats treated to the aqueous extract of leaves of *S. siamea* to the same dose, the report was the same with an increase of 26.53 times 24 h after. This increase persisted and reached on the 14th day value of 550 ± 12.4 U.I/L is 36.67 times J₀. Two weeks after the stop of the treatment and there is a regression that reached a value of 385 ± 11.5 UI/L (25.67 times J₀). For the

rats treated in the mixture of leaves of *S. siamea* and roots of *S. latifolius* (1/1), there was an increase of 9.82 times J₀ 24 h after the treatment, this increase appreciably and reached a value of 310.2 ± 52.2 UI/L (10.34 times J₀). Two weeks after the treatment, this value regressed and reached 135 ± 11.5 UI/L (4.5 times J₀).

About the ALAT, Fig. 3 revealed, an increase of 3.5 and 3.3 times J₀, 24 h after the treatment respectively at the rats treated to the aqueous extracts of roots of *S. latifolius* and leaves of *S. siamea*. This increase persisted and reached his/her/its pick on the day J₁₄ with values 262.0 ± 16.0 UI/L and 235 ± 15.2 UI/L respectively at the rats treated with aqueous extract of roots of *Salat* and leaves of *Sesia*. Two weeks after the end of the treatment (J₂₈), there was a regression that reached respective values of 140.8 ± 2.1 UI/L (3.5 J₀) and 150.8 ± 2.7 UI/L (3.1 J₀). To the dose 2000 mg/kg, we observed that the extracts had all provoked a dizzy increase of the transaminases. This increase was least at the rats treated at the extracts of the *S. siamea* - *S. latifolius* mixture and two weeks after the end of treatment, the rate of the AST fell considerably.

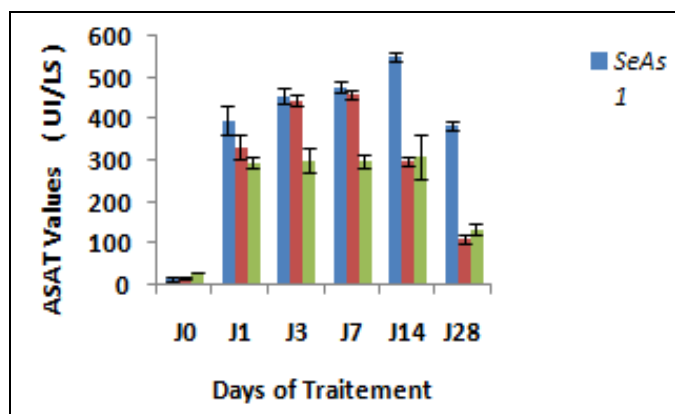


FIG. 2: EVOLUTION OF THE AST AT THE RATS CALLED RESPECTIVELY TO THE DOSE 2000 mg/kg THE AQUEOUS EXTRACT OF LEAVES OF *SESIA*, ROOTS OF *SALAT* AND THEIR MIXTURE. SeAS1: AST value of *S. siamea* leaves, SaAS1: AST value of *S. latifolius* roots, SeSaAS1: AST value of mixture *S. siamea* leave and *S. latifolius* roots

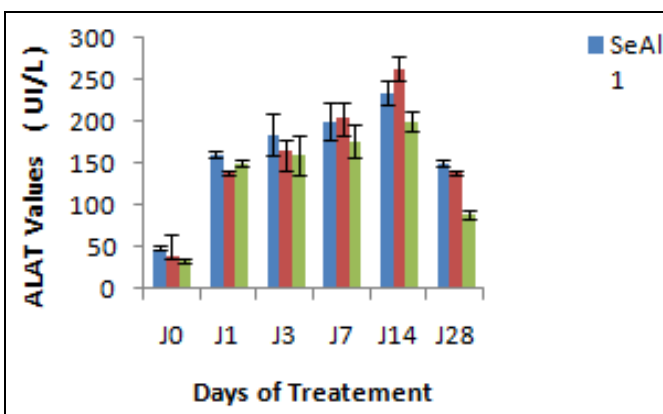


FIG. 3: EVOLUTION OF THE ALT AT THE RATS CALLED RESPECTIVELY TO THE DOSE 2000 mg/kg THE AQUEOUS EXTRACTS OF LEAVES OF *SESIA*, ROOTS OF *SALAT* AND THEIR MIXTURE. SeAl1: ALT value of *S. siamea* leaves, SaAl1: ALT value of *S. latifolius* roots, SeSaAl1: ALT value of mixture *S. siamea*_leave and *S. latifolius* roots

Evolution of Uremia and Creatinemia on Treated Rats: To the dose 2000 mg/kg of aqueous extracts of roots of *S. latifolius*, of the leaves of *S. siamea* and their mixture, we observed, at the treated rats, a meaningful increase of the uremia of 1, 83; 2, 6 and 2.42) since the 3rd day of the

treatment equivalent to respective values of 1.1 ± 0.0; 1.3 ± 0.0 and 0.97 ± 0.1 g/L. This increase remained obstinate until the 14th day of treatment with respective values of 3.0 ± 0.3; 3.5 ± 0.3 and 2.61 ± 0.1 g/L. It is necessary to notice that already on the 7th day the rate of urea was 4.67; 5.8 and 4.5

times the rate of J_0 at the rats treated respectively to the roots of *S. latifolius*, the leaves of *S. siamea* and their mixture. Two weeks after the stop of the treatment (J_{28}), we observed a reduction that brings back the values respectively to $2 \pm S 00$; $2,4 \pm 00$ and 1.58 ± 0.4 g/L for the rats treated to the aqueous extract of roots of *S. latifolius*, the leaves of *S. siamea* and their mixture. The creatinine increased very quickly and reached on the 14th day, 56.0 ± 2.0 ; 60.0 ± 1.1 et 26.0 ± 2.0 mg/L is 4.48; 5

and 2,38 times J_0 at the rats treated respectively to the aqueous extracts of roots of *S. latifolius*, the leaves of *S. siamea* and their mixture. Two weeks after the stop of the treatment (J_{28}), we observed, a meaningful reduction, the creatinémie reached the values of 20.7 ± 1.3 mg/L, 30 ± 2.1 and 12.0 mg/L at the rats treated respectively to the aqueous extracts of roots of *S. latifolius*, the leaves of *S. siamea* and their mixture.

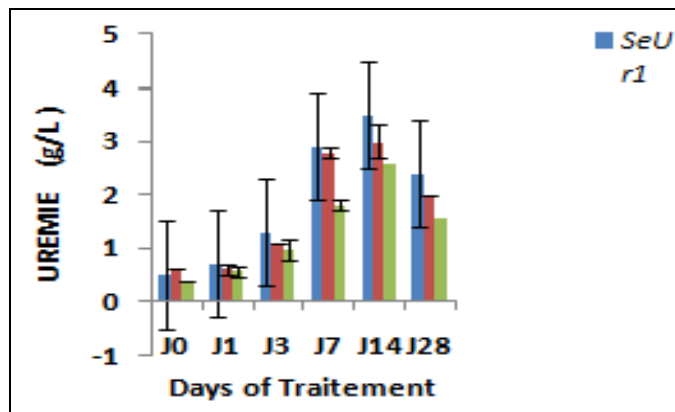


FIG. 4: EVOLUTION OF THE UREMIA AT THE RATS CALLED RESPECTIVELY TO THE DOSE 2000 mg/kg THE AQUEOUS EXTRACT OF LEAVES OF SESIA, ROOTS OF SALAT AND OF THE SESIA - SALAT MIXTURE. SeCr1: creatinémie of *S. siamea* leaves, SaCr1: creatinémie of *S. latifolius* roots, SeSaCr1: creatinémie of mixture *S. siamea* leave and *S. latifolius* root.

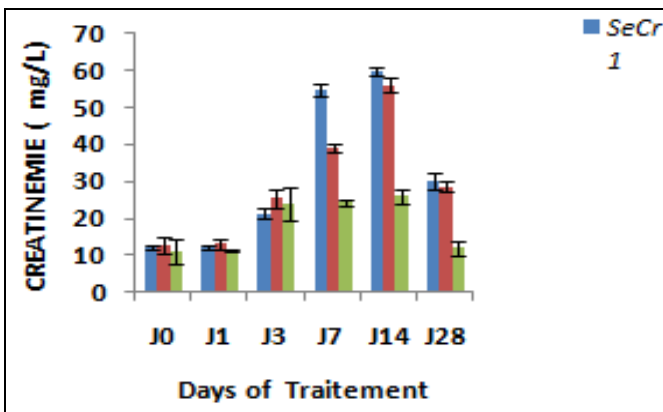


FIG. 5: EVOLUTION OF THE CREATINEMIE AT THE RATS CALLED RESPECTIVELY TO THE DOSE 2000 mg/kg THE AQUEOUS EXTRACT OF LEAVES OF SESIA, ROOTS OF SALAT AND OF THE SESIA - SALAT MIXTURE. SeUr1: uremia of *S. siamea* leaves, SaUr1: uremia of *S. latifolius* roots, SeSaUr1: uremia of mixture *S. siamea* leave and *S. latifolius* roots

These results were confirmed by histological analysis which proved that the renal cortex of the rats treated with the aqueous extract of leaves of *S. siamea* to the dose 2000 mg/kg presented a disorganization of the structure of the renal cortex that become hardly recognizable with a hyalinisation of the regions glomerular giving a picture of " kidney in bread to seal " on the 7th day of treatment.

532ng/ml; 4120 ng/ml and 4000 ng/ml respectively at the rats treated to the aqueous extract of leaves of *S. siamea*, of roots of *S. latifolius* and their mixture.

Evolution of the Concentration of Urinary NGAL at the Treated Rats: The Fig. 6 below showed the curves translating the urinary NGAL expression in the time of the rats treated respectively to the aqueous extract of roots of *S. latifolius* and of leaves of *S. siamea* presented the same pace. These curves have an ascending pace that reaches 2h after the treatment, the values of 3281 ng/ml; 1660ng/ml and 1500ng/ml respectively at the rats treated to the aqueous extract of leaves of *S. siamea* of roots of *S. latifolius* and their mixture. These values increased and reached their picks at the 6th hrs with

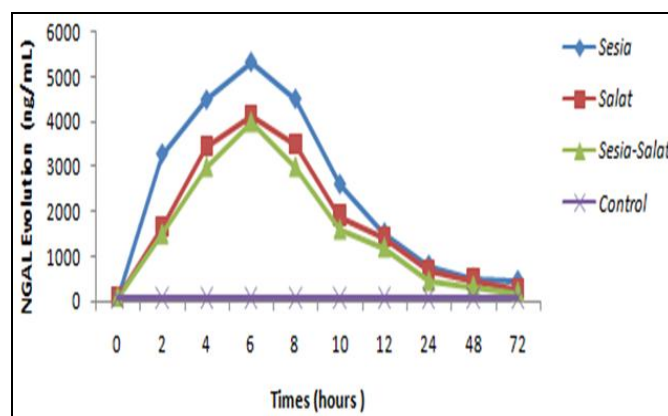


FIG. 6: EVOLUTION OF THE URINARY CONCENTRATION OF NGAL AT THE WITNESS AND THE RATS TREATED TO THE DIFFERENT EXTRACTED OF PLANTS. (n = 5)

We observed a significant regression of these values of the 8th hrs at the 12th hrs ($p = 0.0002$ on average). This regression was no meaningful of the 24th the 72nd h with respective values of 456 ng/ml;

260 ± 0 ng/ml and 200 ng/ml at the rats treated to the extract of leaves of *S. siamea*, of roots of *S. latifolius* and the mixture. The concentrations of NGAL to the level of the rats witnesses evolved around a middle value of 68.7ng/ml.

The histological cut of some organs appropriated at the rats milked respectively to the dose of 2000 mg/kg of the aqueous extract of leaves of *S. siamea*, of roots of *S. latifolius* and the mixture. To-normal histological different studied organ aspect.

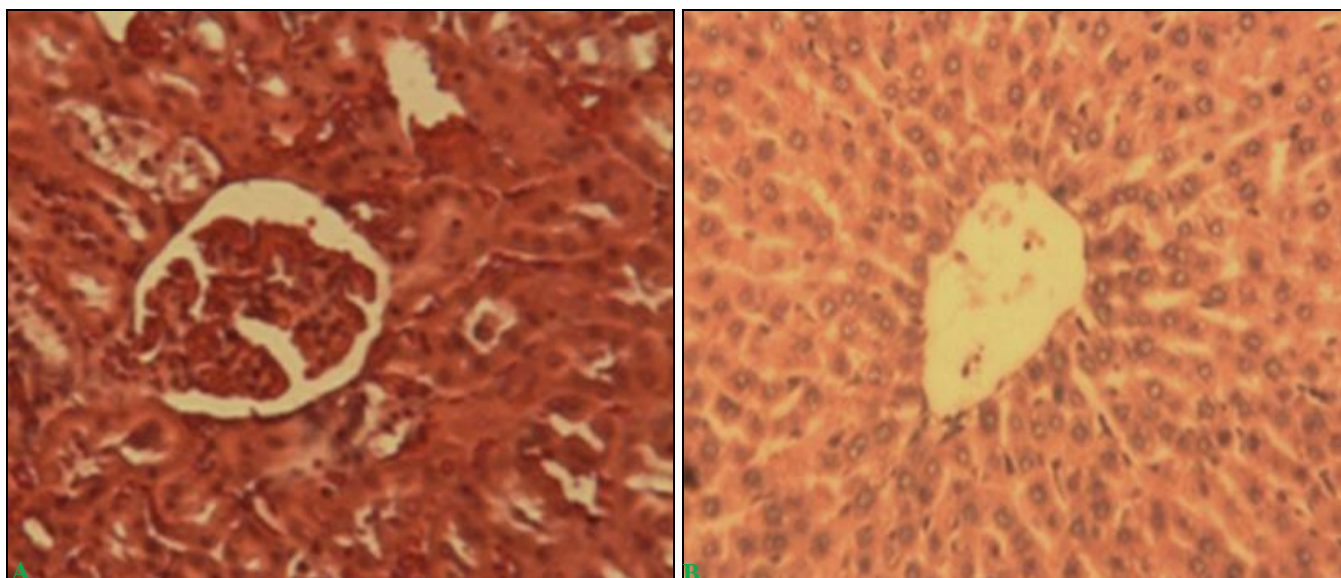


FIG. 7: NORMAL HISTOLOGY OF THE LIVER AND THE RENAL CORTEX OF WISTAR RAT. A) NORMAL RENAL CORTEX OF WISTAR RAT SHOWING A RENAL GLOMERULAR (G X 100). B) NORMAL HEPATIC LOBULE OF WISTAR RAT (G X 100)

The histological aspects of the renal cortex of rat wistar after sharp administration of the dose 2000 mg/kg respectively of the aqueous extract of leaves of *S. siamea* and roots of *S. latifolius* are presented in the **Fig. 8A** and **8B**. The **Fig. 9A** and **9B** show the histological aspects of the liver of Wistar rat respectively after sharp administration of dose 2000 mg/kg of the aqueous extract of leaves of *S. siamea*

or roots of *S. latifolius*. The administration of the aqueous extract of leaves of *S. siamea* to the dose 2000 mg/kg creates some signs of necroses at the rats that the level of the renal cortex that hepatocyte what was not observed at those treated to the aqueous extract of roots of *S. latifolius* to the same dose.

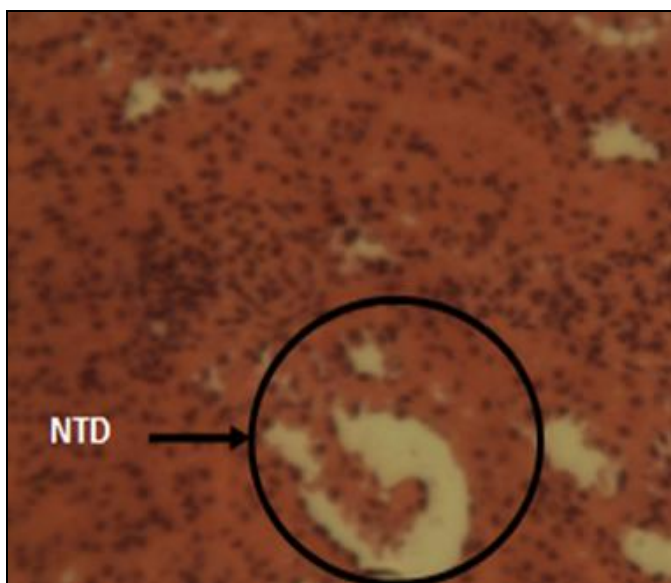


FIG. 8A: RENAL CORTEX OF RAT SUBMITTED TO A UNIQUE DOSE OF 2000 mg/kg OF SESIA (G X 100)

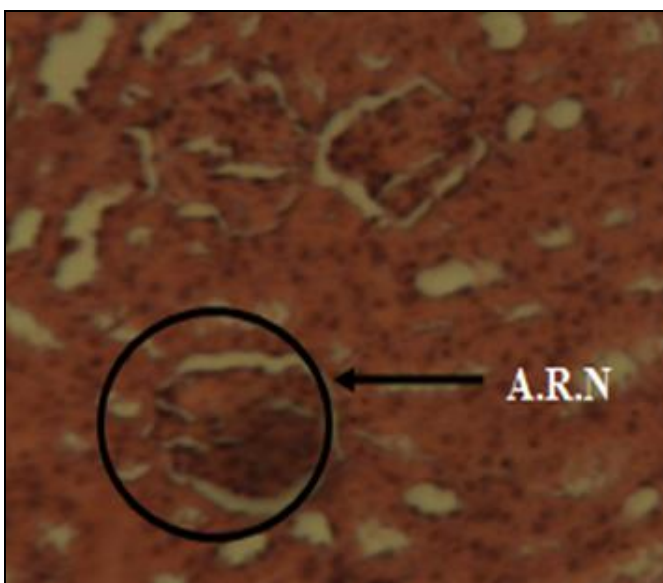


FIG. 8B: RENAL CORTEX OF RAT SUBMITTED TO AN UNIQUE DOSE OF 2000 mg/kg OF SALAT (G X 100)



FIG. 9A: LIVER OF RAT SUBMITTED TO A SINGLE DOSE OF 2000 mg/kg OF LEAVES OF SESIA: (GX 100)

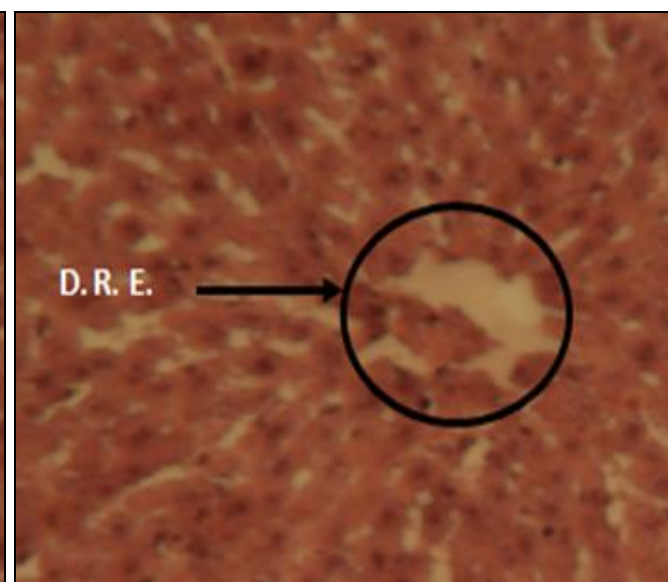


FIG. 9B: LIVER OF RAT SUBMITTED TO A SINGLE DOSE OF 2000 mg/kg OF ROOTS OF SALAT SHOWING AN ALMOST NORMAL HISTOLOGICAL ASPECT (GX 100), SESIA: (GX 100)

Sub-Acute Toxicity: Histological impacts of the treatment of the rat's Wistar to the dose of 2000 mg/kg of body weight of the aqueous extract of leaves of *S. siamea* and roots of *S. latifolius* during 7 days.

The Liver Aspects: The Fig. 10A and 10B show the histological aspects of the liver of rat wistar after 7 days of treatment to the lethal dose of 2000 mg/kg of body weight of the aqueous extract of leaves of *S. siamea* and roots of *S. latifolius*.

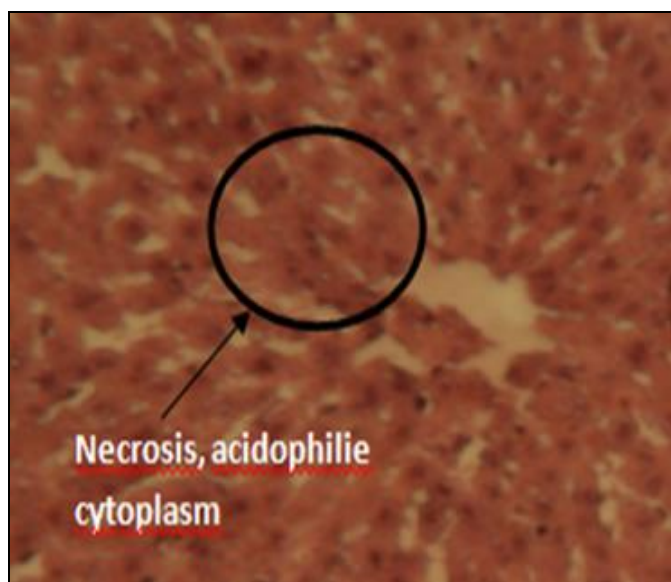


FIG. 10A: LIVER OF RAT TREATY DAILY WITH 2000 mg/kg OF BODILY WEIGHT OF LEAVES OF SESIA DURING 7 DAYS (G X 100)

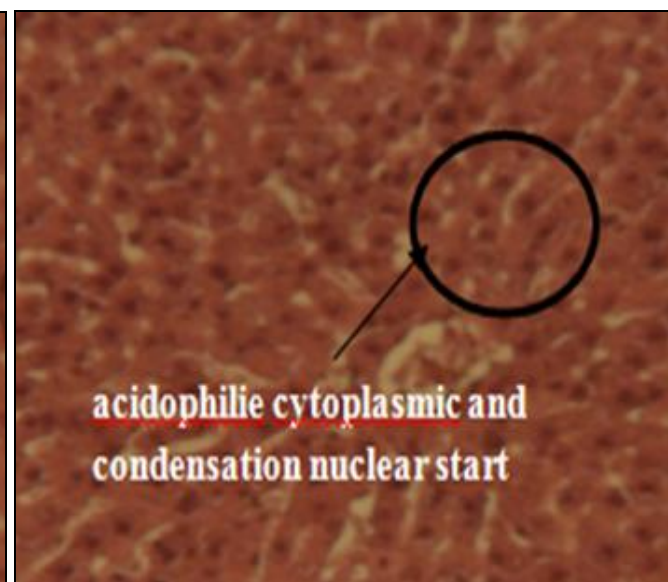


FIG. 10B: LIVER OF RAT TREATY DAILY WITH 2000 mg/kg OF BODILY WEIGHT OF ROOTS OF SALAT DURING 7 DAYS: HEPATIC ARCHITECTURE PRESERVED BUT (G X 100)

Renal aspects: The histological aspects of the renal cortex of wistar rat after 7 days of treatment at the daily dose of 2000 mg/Kg of body weight for aqueous extract of *S. siamea* leaves and *S. latifolius* roots were presented in Fig 11A and 11B. The

aqueous extract of leaves of *S. siamea* to the dose 2000 mg/kg destroyed the renal cortex and created of necrosis to the level hepatocyte with pycnoses of the core.



FIG. 11A: RENAL CORTEX OF RAT TREATED DAILY WITH 2000 mg/kg OF BODY WEIGHT OF LEAVES OF *SESA* DURING 7 DAYS (G X 100)



FIG. 11B: RENAL CORTEX OF RAT TREATED DAILY WITH 2000 mg/kg OF BODY WEIGHT OF ROOTS OF *SALAT* DURING 7 DAYS (G X 100)

DISCUSSION: To guarantee the innocuity of the products destined to the man in the conditions of jobs foreseen, some toxicological studies must be achieved on laboratory animals. The different tests of toxicity permitted to determine the Lethal Dose 50 % (LD_{50}) of leaves of *S. siamea*, of roots of *S. latifolius*, and the one of their mixture. The LD_{50} of these different plants and mixture were superior to 2000 mg/kg, our results corroborated those of Mohammed and coll.¹⁶ that indicated that the Lethal Dose₅₀ of the aqueous extract of *S. siamea* was superior to 5000 mg/kg. The aqueous extract of leaves of *S. siamea* was slightly more poisonous than the one of roots of *S. latifolius* and of the mixture of *S. siamea* leaves - *S. latifolius* roots with as lethal concentrations (LC_{50}) respective of 0,78; 1,56, and 3,16 mg/ml.

According to the scale of toxicity of Hodge and Sterner¹⁷ and of the WHO¹⁸, our extracts were weakly poisonous and classified in the III class of the chemical substances.

According to José and coll.¹⁹, an interrelationship existed between the toxicity of the shrimp larvae (*Artemia salina*) and pulmonary cell toxicity. The larvae of shrimp pickle were sensitive to the poisonous effects of the aqueous extracts of leaves of *S. siamea*, of roots, and the one of the mixtures of *S. siamea* leaves - *S. latifolius* roots. The lethal concentrations gotten at the shrimp larvae pickle were too weak to predict toxicity at the man.

Several poisonous plants compound accumulated in the liver where they are detoxified²⁰. The liver was the first target of the toxicity and the first organ exposed to everything that is absorbed in the spindly intestine; he/it metabolizes the substances foreign to compounds that can be hepatotoxic²¹. The liver worked in association with the kidneys to suppress the poisonous substances of blood²².

A survey of the renal and hepatic function can prove to be therefore useful to value the poisonous medicinal plant effects. These tests consisted mainly of the determination of the AST, the ALT, the creatinemy, the urea and other²³ and all hepatic cell necrosis derived to a meaningful rise of the AST enzymes, ALT in the blood serum²⁴.

The ALT and the AST were the cytotoxic enzymes whose rates rise quickly when the liver was damaged for various reasons including the hepatic cellular necroses, hepatitis, the cirrhosis as well as the hepatotoxicity of some drugs²⁵.

The seric studies done on the rats treated by the aqueous extract of leaves of *S. siamea*, of roots of *S. latifolius* and their mixture to the dose 2000 mg/kg showed a meaningful increase of the parameters: ASAT, ALAT, the urea and the creatinemy in relation to the witnesses These increases would translate a reach hepatocytary²⁶. This reach has been confirmed, by the reduction of the spaces vascular and some signs of hepatocytary

necrosis in the centrolobular region **FIG. 9A**. This poisonous action could be assigned to the different secondary metabolites as the saponosides, the tannins, the flavonoïdes, present in the aqueous extract respectively of the leaves of *S. siamea* and roots of *S. latifolius* as Agbaje and coll,²⁷ suggested it, at the time of the survey of the toxicity subaigüe of *Syzigium aromaticum* on the rats.

To the level of the rats treated to the dose 2000 mg/kg of aqueous extract of leaves of *S. siamea*, there was a disorganization of the structure of the renal cortex that become hardly recognizable with a hyalinisation of the glomerular regions giving a picture of " kidney in bread to seal " on the 7th day of treatment **Fig. 11A**.

Of this fact, he/it was probable that the aqueous extracts of the leaves of *S. siamea*, of the roots of *S. latifolius* and of their mixture, misled a vascular proliferation to the level of the centrolobular zone and the space carried from the successive metabolisms bound to the daily stuffings to single dose. The successive increases of the rates of the transaminases found all their explanations in the numerous metabolism complexities that took place after the stuffings of the animals to the extracts of plants in the case of experimentation.

In all aggressions by the xenobiotical, the hepatic metabolism of the proteins was modified toward the production of the defense systems and the neoglucogenesis. The deterioration of the compounds proteic hepatic could explain the increase of the urea and seric creatinin at the treated rats, where the proteins can be damaged then in amino acids in urea and creatinin. So these formed amino acids could turn under the action of the seric transaminases into carboxylic compounds as the pyruvic acidic²⁸. It implies the strong enzymatic activity of the transaminases.

The seric rates of creatinine and urea that was of excellent scorers of the renal function are generally constant in the normal conditions²⁹. The increase or the reduction of these scorers reflected a renal dysfunction³⁰. The interest of the measure of the rate of the urea, consisted in valuing the intensity of the protidic destruction, herself reflection of the lesions associated to all metabolic process. The

changes can reach the numerous structures of the kidneys in a reversible or chronic way.

The results of this survey permitted to appreciate the exhibition of the population to the medicinal plant use. It puts the problematic of the security and the health of the Beninese population. These different arrangements were not sufficient to confirm the toxicity of the extracts of the leaves of *S. siamea*, of the roots of *S. latifolius* and the mixture.

In the animal model, the NGAL was one of the proteins, induced the most precociously after a renal reach of ischemic type or poisonous³¹. He is used in different situations clinics as in postoperative of cardiac surgery, at the time of a renal transplantation³², after an administration of products of contrasts iodized or in intensive care. A concentration of NGAL in urine above 350 ng/ml or in plasma 400 ng/ml was above strongly likely (> 90 %, positive value predictive) to be due to the renal damages that can lead to the renal failure. A sudden elevation of the urinary concentration of NGAL above the patient's previous values can indicate the renal damages even though the value limits is not passed, and the narrow surveillance of the renal function is recommended.

The dosage of NGAL in this survey has for objective to detect precocious manner the effect of the administration by oral way of the aqueous excerpt of leaves of *S. siamea*, of roots of *S. latifolius* and their mixture to the dose 2000 mg/kg. The gotten results showed a dizzy rise of the urinary NGAL rate **Fig. 6** enters 0 and 6 h whatever is the extract, there is a meaningful difference respectively between the rate of NGAL of the three extracts of plants at the time 2 h, 4 h and 6 h.

The rate raised from NGAL could signal damages of the kidney³³. This result confirms those especially gotten on the biochemical and histological side for the aqueous extract of *Sesia*.

Of this fact, we will say that the dose 2000 mg/kg of these different extracts apparently without effect on motility, the lucidity of the animals, create some damages to the levels of the organs as the liver and the kidneys.

CONCLUSION: The toxicity of the aqueous extract of leaves of *Senna siamea*, of roots of *Sarcocephalus latifolius* and their mixture (1/1) to the dose 2000 mg/kg has been performed. Information pulled from the set of the results presented in this work on the applied data of the acute toxicity at the rats Albino wistars suggested classifying leaves of *Senna siamea*, of roots of *Sarcocephalus latifolius* and their mixture in the weakly toxicity plant's category by the oral way.

However, the sub-acute toxicity showed that the aqueous extract of these plants and their mixture (1/1) at 2000 mg/kg disrupts some biochemical parameters bound to the hepatic, renal and muscular function. The histological observation was especially characterized by structural changes of the liver and the kidney on the 7th day. These changes were confirmed by the rate raised from NGAL in the urine, noted since the first hours of the rats treated. The further works will be focused on the pharmacological activities and toxicological on human cells studies for moderation of security in the use of these plants.

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CONFLICT OF INTEREST: Nil

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