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Evaluation of the Liver Toxicity and Nephrotoxicity of Creatinor, a Natural Recipe Used in the Treatment of Renal Failure in Côte d'Ivoire

Irié Bi Tra Olivier[1]*, Ekissi Yapi Huges Romaric[2], N'doua Akouah Richmonde Leatitia[3], Djaman Allico Joseph[4] and Abo Kouakou Jean-Claude[1]

1.Laboratory of Biology and Health, Specialty Animal Physiology, Phytotherapy and Pharmacology, Biosciences Training and Research Unit (UFR), Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire.

2.Laboratory of Animal Biological Sciences, Specialty Animal Physiology, Phytotherapy and Pharmacology, Unit of Training and Research (UFR) Science and Technology, Alassane Ouattara University, Bouaké, Côte d'Ivoire.

3.Laboratory of Biodiversity and Tropical Ecology, Specialty Animal Physiology and Pharmacology, University Jean Lorougnon Guede, Daloa, Côte d'Ivoire.

4. Department of Medical and Fundamental Biochemistry, Institute Pasteur in Côte d'Ivoire.

ABSTRACT

Creatinor is a natural recipe used in Côte d'Ivoire by traditional therapists to treat kidney failure. However, its toxicity has not yet been scientifically studied. The aim of this study is to assess the safety threshold of Creatinor use in mice and the toxic potential of this substance on the kidneys and liver of healthy rats. The acute oral toxicity study of Creatinor, in accordance with OECD 423 recommendations, shows that this substance is non-toxic in mice. The sub-acute toxicity study of Creatinor in healthy rats, in accordance with OECD guideline 407, showed that doses of 100 and 200 mg/kg bw caused, in a dose-dependent manner, decreases in the relative weights of the kidneys and liver of treated rats and, from the 21st day of treatment, increases in the levels of creatinine, urea, ALAT and ASAT. Creatinor also induced disorganisation of the histological structures of the kidneys and liver, with tissue necrosis. Creatinor, administered to healthy rats over a long period at doses of 100 and 200 mg/kg bw, is said to have nephrotoxic and hepatotoxic effects. At 200 mg/kg bw, these effects were more marked and more precocious, with adverse effects on body development. On the other hand, Creatinor at 50 mg/kg bw has no effect.

Key words: Creatinor, toxicity, nephrotoxicity, hepathotoxicity, renal failure

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INTRODUCTION

In many countries around the world, people use natural recipes to face their health needs. In developing countries, medicinal plants are the most widely used means of solving health problems. In Africa, some 80% of the populations rely on traditional medicine to meet their basic health needs (1) Herbal medicines are often mistakenly considered safe because they are "natural". Nevertheless, these products contain bioactive principles that can cause adverse effects (2). Thus, the WHO recommends the evaluation of the safety and efficacy of herbal medicines with a view to standardizing their use and integrating them into conventional healthcare systems (3). In Côte d'Ivoire, a natural recipe called "Creatinor" is used by traditional therapists to treat kidney failure. However, its toxicity has not yet been scientifically studied. The aim of this study is therefore to assess the safety threshold of Creatinor use in mice, and the toxic potential of this substance on essential organs such as the kidneys and liver of healthy rats.

MATERIALS AND METHOD

Plant material

The material used is a liquid preparation based on codified medicinal plants called Creatinor. It is offered to patients suffering from renal failure in a phytotherapy practice in Côte d'Ivoire. This solution is filtered, then freeze-dried to obtain a dark-brown powder with a pleasant odor and bland taste. The lyophilisate is stored in a bottle in the freezer at -4°C for the duration of the experiment.

Animal material

Female mice, Mus musculus (Muridae), are used for the acute toxicity study. These mice are 4 weeks old and weigh between 20 and 25 g. Male and female Wistar rats, Rattus norvegicus (Muridae), are used to assess subacute toxicity. They weigh between 175 and 240 g and are between 3 and 4 months old. These animals are bred under standard laboratory conditions and maintained in a 12 h light-dark cycle, with a temperature of 24-26°C and a humidity of 54-60%, and have free access to water and food consisting of pellets (Ivograins®, Abidjan). All experimental protocols are conducted in compliance with the European Directive of November 24, 1986 (86/609/EEC) and the Decree of April 19, 1988 on the use of experimental animals (4).

Methods

Creatinor acute toxicity study methods

The Creatinor acute toxicity study was carried out orally in accordance with OECD 423 guidelines (5) on thirty-six one month-old female mice, Mus musculus (Muridae), weighing between 20 and 25 g. This study consisted of testing single doses of 5, 50, 300, 2000 and 5000

mg/kg bw of Creatinor. This study involved testing single doses of 5, 50, 300, 2000 and 5000 mg/kg bw of Creatinor. Mice are divided into six batches (5 tests batches and 1 control batch) of six. After withholding food for 4 h, but not water, the control group receives 2 ml of distilled water, and animals in batches 2, 3, 4, 5 and 6 receive 2 ml of 5, 50, 300, 2000 and 5000 mg/kg bw Creatinor respectively. After treatment, the animals are fed and have free access to water. The effects on the behaviour and morphology of treated animals were observed for 24 hours, with particular attention paid to the first 4 hours, and daily thereafter, for a total of 14 days, and symptomatic toxicity disorders in these animals were noted. The number of dead mice was counted 48 hours after injection and then repeated after 14 days.

Study methods for Creatinor sub-acute toxicity

The Creatinor sub-acute toxicity study was conducted in rats in accordance with OECD guideline 407 (6). It was conducted on twenty-four eight-week-old Wistar rats, divided into four equal batches of 3 males and 3 females. The doses tested ranged from 50 to 200 mg/kg bw. They were based on a dose of 100 mg/kg bw, which is the dose used by traditional therapists in the treatment of renal failure in Côte d'Ivoire. Rats in the control group were given distilled water at a rate of 1 ml per 100 mg/kg bw, and rats in groups 1, 2 and 3 were given a Creatinor solution at a rate of 50, 100 and 200 mg/kg bw respectively. These Creatinor doses were chosen according to the dose used by the practitioner (100 mg/kg bw). Treatments lasted 28 days. The rats are fed and have unlimited access to water. The animals were weighed every week and blood samples were taken to measure biochemical parameters (urea, creatinine, alanine aminotransferase, aspartate aminotransferase) using an automated system (Rayto chemray 120, China). During the 28-day experiment, observations were made on the animals' behaviour and morbidity. At the end of the experiment, the animals were sacrificed using ketamine at a dose of 50 mg/kg bw. and histological sections were taken from the vital organs, (kidney and liver), which were weighed before being observed under an optical microscope (MT4000 Meiji Techno, France). The relative weights of these organs were evaluated using the following formula:

Pr = (PO/PA) X100

Pr: relative weight (g/kg bw)

PO: organ weight (g)

PA: average weight of animals per batch (kg)

Biochemical and haematological analysis methods

Blood was collected at the start of treatment (D0) and every week (D7, D14, D21 and D28) from the caudal artery of the rats. Blood samples were collected in dry tubes to measure serum levels

of urea, creatinine, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT). The blood sample is centrifuged at 4,000 rpm at 4°C for 5 minutes. The serum obtained is aliquoted and stored at -20°C until it is analysed. These analyses are carried out using an automatic biochemical analyzer (Rayto chemray 120, China).

Histological study method

Rat liver and kidney were exposed by laparotomy and excised, samples of the different tissues are fixed in 10% paraformaldehyde (0.1 Phosphate saline buffer) at room temperature for 48 dehydrated, processed, and then embedded in paraffin. Paraffin sections of each tissue are cut to a thickness of 5 µm using a microtome (RMT-SA390, China) and stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS) and Jones methenamine silver, following conventional staining protocols **(7).** The microscopic slides were observed and analysed using a light microscope (Olympus, France), (Magnifications: x 5 and x 10).

Statistical study

Statistical data processing was carried out using GraphPad Prisme 8.4 software (Microsoft, San Diego, California, USA). Values are expressed as the mean plus the standard error of the mean (M ± SEM). Descriptive statistics are used to calculate the mean of different parameters. Tukey's test is used for pairwise comparisons of means. Dunnet's test is used to compare the mean of each group with the control group. In all statistical tests, the significance threshold is set at 0.05, with: insignificant for $p < 0.05$ (*), significant for $p < 0.01$ (**) and highly significant for $p < 0.001$ (***).

RESULTS AND DISCUSSION

Evaluation of the acute toxicity of Creatinor in mice

Behaviour of mice after oral administration of single doses of Creatinor

After oral administration of single, predefined doses of 5, 50, 300, 2000 and 5000 mg/kg bw Creatinor to mice, observation of clinical signs such as locomotion, respiration, tremor, mobility, stool appearance, etc. for 3 hours showed no symptomatic toxicity in these animals. In addition, these animals drank and ate normally. Thus, the behaviour and physiology of these treated mice are normal, identical to those of control mice.

Mortality of mice after oral administration of single doses of Creatinor

No deaths of mice were obtained following administration of Creatinor at single doses of 5, 50, 300, 2000 and 5000 mg/kg bw, after 24 h observation, and even after 14 days.

Evaluation of the subacute toxicity of Creatinor in rats

Dose-response effects of Creatinor on body weight in healthy rats treated for 28 days

The average weight of healthy rats before treatment (at D0) was 285 ± 2.57 g. This weight increased slowly and steadily each week, but not significantly ($p > 0.05$), until day 28. The increases were 2.3%, 4.7%, 8.3% and 11.8% on days 7, 14, 21 and 28 respectively. In rats treated for 28 days with Creatinor at a dose of 50 mg/kg bw, body weight increased steadily but not significantly ($p > 0.05$) and similarly to control rats, from 280.5 ± 2.85 g at D0 to 294.7 ± 2.84 g at D28 ; an increase of 5.06%. On the other hand, with the 100 mg/kg bw. Creatinor dose given by gavage to the rats over 28 days, the weight of these animals did not decrease significantly (p > 0.05) from 286.8 ± 1.82 g on D0 to 280.8 ± 0.95 g on D28, i.e. a decrease of 2.03%. In rats given a dose of 200 mg/kg bw. Creatinor, body weight decreased slightly over the 28 days of experimentation compared with the weight of healthy control rats. This decrease became insignificant ($p < 0.05$) from the 21st day of treatment when the weight of these animals fell from 282.2 ± 1.78 g on D0 to 229 ± 0.22 g on D21 and 219.2 ± 4.1 g on D28, i.e. decreases of 18.85% and 22.32% respectively (Figure 1)

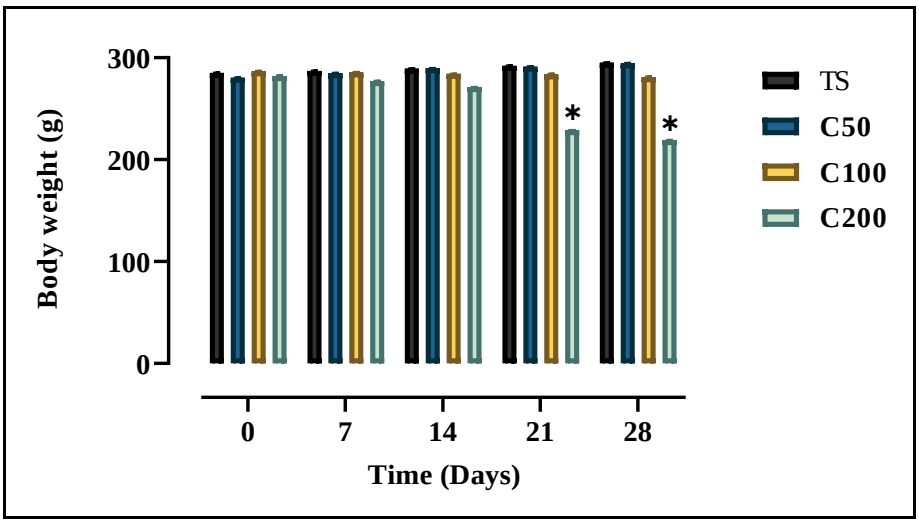

Figure 1: Changes in body weight in healthy rats treated for 28 days with Creatinor

*Values are expressed as the mean, followed by the standard error of the mean (M ± SEM), n = 6 ; * p < 0.05 compared with controls.*

TS : Control rats

C50: Rats treated with Creatinor at 50 mg/kg bw, C100: Rats treated with Creatinor at 100 mg/kg bw., C200: Rats treated with Creatinor at 200 mg/kg bw.

Dose-response effects of Creatinor on relative kidney weight in healthy rats treated for 28 days

Before the study, the relative kidney weight of control rats was 1.71 ± 0.01 g/kg bw. It did not vary significantly ($p > 0.05$) during 28 days of experimentation. When 50 mg/kg bw Creatinor was administered to the rats, their relative kidney weight did not vary significantly (p > 0.05) ;

this relative weight fell from 1.71 ± 0.01 g/kg bw on D0 to 1.63 ± 0.08 g/kg bw on D28, i.e. a 4.8% reduction. Gavage of rats with 100 mg/kg bw Creatinor also resulted in a decrease in relative kidney weight from 1.71 ± 0.01 g/kg bw at D0 to 1.01 ± 0.09 g/kg bw at D28. This decrease in relative weight represents a marginally significant ($p<0.05$) reduction of 41.1%. The relative kidney weight of rats decreased progressively when Creatinor 200 mg/kg bw was administered. This reduction was significant ($p < 0.01$), as the relative weight fell from 1.71 ± 0.01 g/kg bw at D0 to 0.70 ± 0.09 g/kg bw at D28, i.e. a 58.92% reduction (**Figure 2**).

Dose-response effects of Creatinor on relative liver weights in healthy rats treated for 28 days

Relative liver weights in control rats did not vary significantly ($p > 0.05$) over the 28-day experimental period. It was 3.5 ± 0.17 g/kg bw. Administration of 50, 100 and 200 mg/kg bw Creatinor to rats resulted in decreases in relative liver weights compared with control rats. When rats were given 50 mg/kg bw Creatinor, their relative liver weight fell from 3.5 ± 0.01 g/kg bw at D0 to 3.2 ± 0.02 g/kg bw at D28, a reduction of 8.57%. This reduction was not significant ($p > 0.05$). The decrease in relative liver weight obtained with Creatinor 100 mg/kg bw administered to rats for 28 days was also not very significant ($p < 0.05$). Relative liver weight fell from 3.5 ± 0.17 g/kg bw at D0 to 2.2 ± 0.3 g/kg bw at D28, corresponding to a reduction of 37.14%. The relative liver weights of the rat animals decreased from 3.5 ± 0.01 g/kg bw at D0 to 1.5 ± 0.04 g/kg bw at D28 after administration of 200 mg/kg bw Creatinor. This corresponds to a significant reduction ($p < 0.01$) of 57.2% (Figure 3).

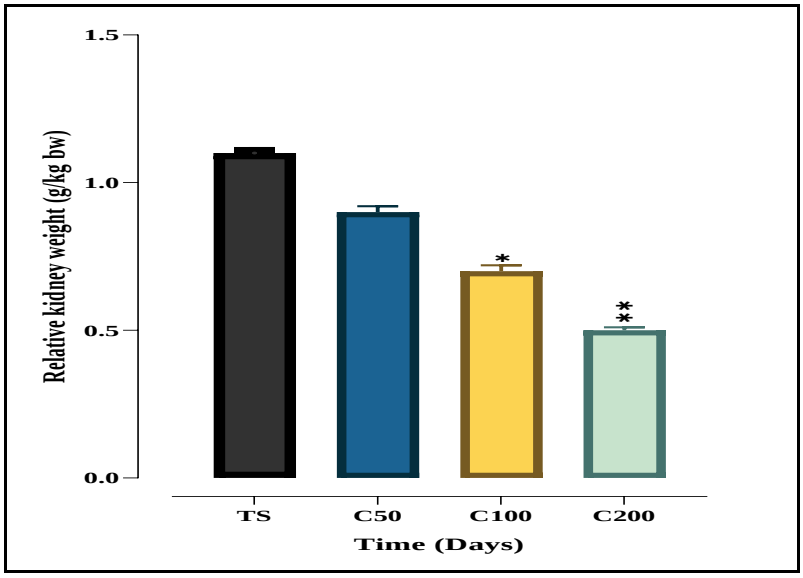

Figure 2: Effects of Creatinor on relative kidney weights in healthy rats after 28 days of treatment

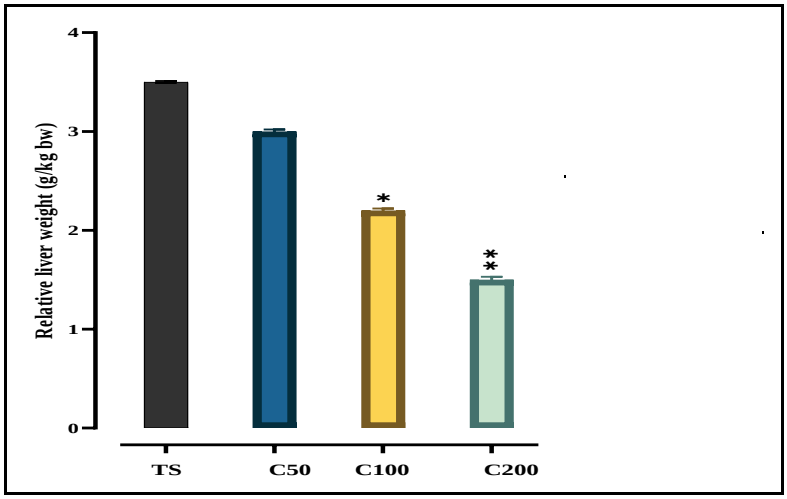

Figure 3: Effects of Creatinor on relative liver weights in healthy rats after 28 days of treatment

*Values are expressed as the mean, followed by the standard error of the mean (M ± SEM) n = 6 ; * p < 0.05; ** p < 0.01 compared to controls*

TS : Control rats , C50 : Rats treated with Creatinor at 50 mg/kg bw., C100 : Rats treated with Creatinor at 100 mg/kg bw., C200 : Rats treated with Creatinor at 200 mg/kg bw

Dose-response effects of Creatinor on serum creatinine levels in healthy rats during 28 days of treatment

Serum creatinine levels in control rats were constant (p > 0.05) during the 28 days of experimentation. This level was 7.85 ± 0.38 mg/dl. By administering 50 mg/kg bw of Creatinor, the creatinine level (7.85 ± 0.13 mg/dl) of the rats remained constant (*p > 0.05*) during the 28 days of experimentation. The serum creatinine level in rats treated with 100 mg/kg bw. Creatinor was 7.15 ± 0.24 mg/dl at the end of 28 days of experimentation, indicating an increase of 6.51% since the level in these animals was 6.68 ± 0.21 mg/dl at D0. This increase was not significant (*p > 0.05*) compared with the serum creatinine level in control rats. At the end of the experiment, the creatinine level of rats given Creatinor 200 mg/kg bw was 44.46 ± 1.73 mg/dl. This shows a highly significant increase (*p<0.001*) of 82.34% in the creatinine level of these rats, as their pre-treatment value was 7.08 ± 0.38 mg/dl (**Figure 4**).

Dose-response effects of Creatinor on serum urea levels in healthy rats during 28 days of treatment

During the 28-day study, urea levels in control rats remained constant (*p > 0.05*) at approximately 0.24 mg/dl. The urea levels of rats given 50 mg/kg B.P. of Creatinor did not vary significantly (*p > 0.05*), rising from 0.24 ± 0.004 mg/dl at D0 to 0.24 ± 0.01 mg/dl at D28. Rats given 100 mg/kg bw Creatinor had a urea level of 0.39 ± 0.02 mg/dl at the end of 28 days of

experimentation. This urea level increased from 0.238 ± 0.28 mg/dl on D0 to 0.39 ± 0.02 mg/dl, representing a marginally significant ($p<0.05$) increase of 38.97%. The increase in urea levels from 0.24 ± 0.01 mg/dl at the start of the experiment to 0.53 mg/dl at the end of 28 days of treatment showed a significant increase ($p < 0.01$) in urea levels in rats given 200 mg/kg bw Creatinor. This increase in urea was 54.71% (Figure 5).

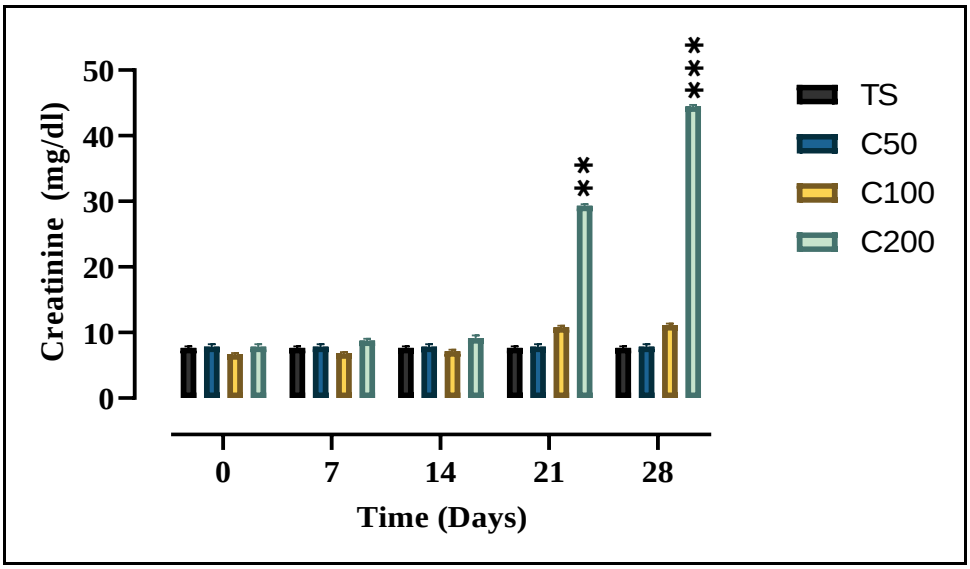

Figure 4: Changes in serum creatinine concentration in healthy rats treated for 28 days with Creatinor

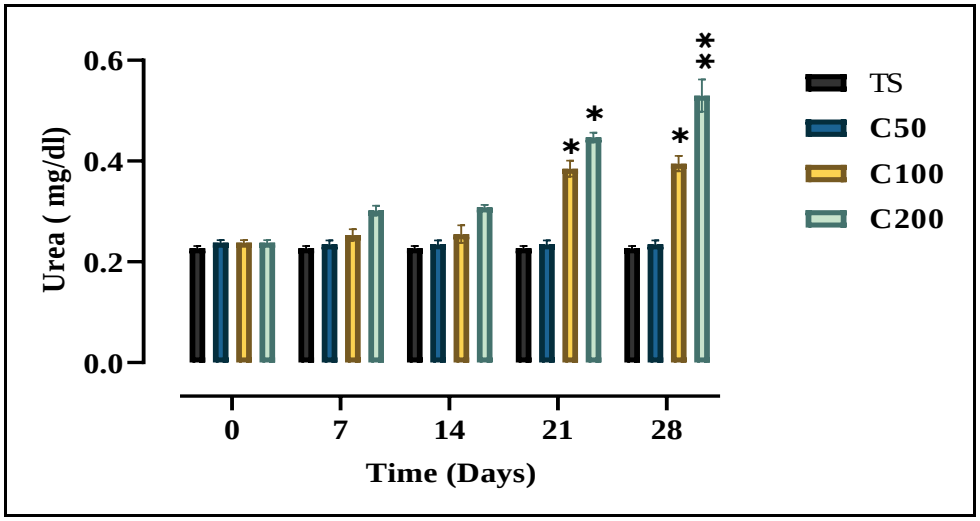

Figure 5: Changes in serum urea concentration in healthy rats treated for 28 days with Creatinor

Values are expressed as mean, followed by standard error of the mean (M ± SEM)

*n = 6 ; * p < 0.05; ** p < 0.01; *** p < 0.001 compared to controls*

TS : Control rats , C50 : Rats treated with Creatinor at 50 mg/kg bw., C100 : Rats treated with Creatinor at 100 mg/kg bw, C200 : Rats treated with Creatinor at 200 mg/kg bw

Dose-response effects of Creatinor on aspartate aminotransferase (ASAT) levels in healthy rats during 28 days of treatment

The ASAT level in rats fed only water (control rats) was 17.87 ± 0.39 IU/L during the 28 days of experimentation. Rats given 50 mg/kg bw of Creatinor had an ASAT level of 17.88 ± 0.29 IU/L on D0 and this level remained constant ($p > 0.05$) until the end of the experiment. The ASAT level of rats treated with 100 mg/kg bw Creatinor was 23.98 ± 0.02 IU/L after 28 days of experimentation (D28). At the start of the experiment, i.e. at D0, this level was 17.85 ± 0.39 IU/L, indicating a 34.2% increase in ASAT levels in rats treated with 100 mg/kg P.C. of Creatinor. This increase was significant ($p < 0.05$). Before the 28th day of treatment, ASAT levels did not vary significantly compared with control rats. In rats given Creatinor 200 mg/kg bw, the ASAT level was 17.87 ± 0.19 IU/L on day D0. This level increased significantly ($p < 0.01$) to 27.98 ± 0.39 IU/L on day 21, then to 33.98 ± 0.01 IU/L ($p < 0.001$) at the end of the experiment, representing increases of 59% and 90.1% respectively (**Figure 6**).

Dose-response effects of Creatinor on alanine aminotransferase (ALAT) levels in healthy rats during 28 days of treatment

ALAT levels in control rats did not vary ($p > 0.05$) during the 28 days of experimentation. It was 14.08 ± 0.03 IU/L. With 50 mg/kg bw of Creatinor administered to the rats, the ALAT level did not also vary and was 15.08 ± 0.03 IU/L, approximately equal to that of the control rats, at the end of the experiment. In rats treated with 100 mg/kg bw Creatinor, the ALAT level was 25.83 ± 0.08 IU/L after 28 days of experimentation. Considering the ALT level at D0, which was 15.85 ± 0.02 IU/L, only on day 28 was there a marginally significant ($p < 0.05$) 38.7% increase in ALT levels in rats given 100 mg/kg bw of Creatinor. The ALT level rose from 15.83 ± 0.03 IU/L (D0) to 23.83 ± 0.04 IU/L ($p < 0.01$) at D21, then increased again to reach 35.83 ± 0.03 IU/L ($p < 0.001$) on day 28 in rats given 200 mg/kg bw of Creatinor. These changes represent 53.33% and 133.33% increases respectively (Figure 7).

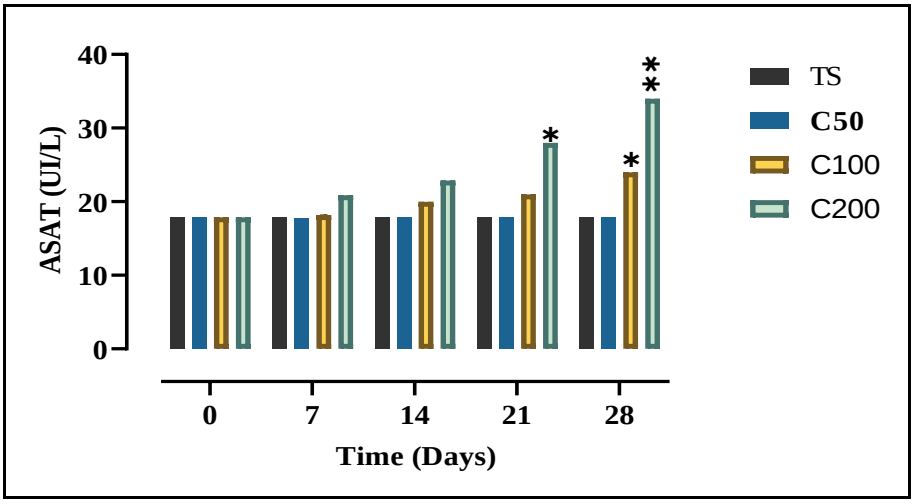

Figure 6: Changes in serum aspartate aminotransferase (ASAT) levels during 28 days of treatment of healthy rats with Creatinor

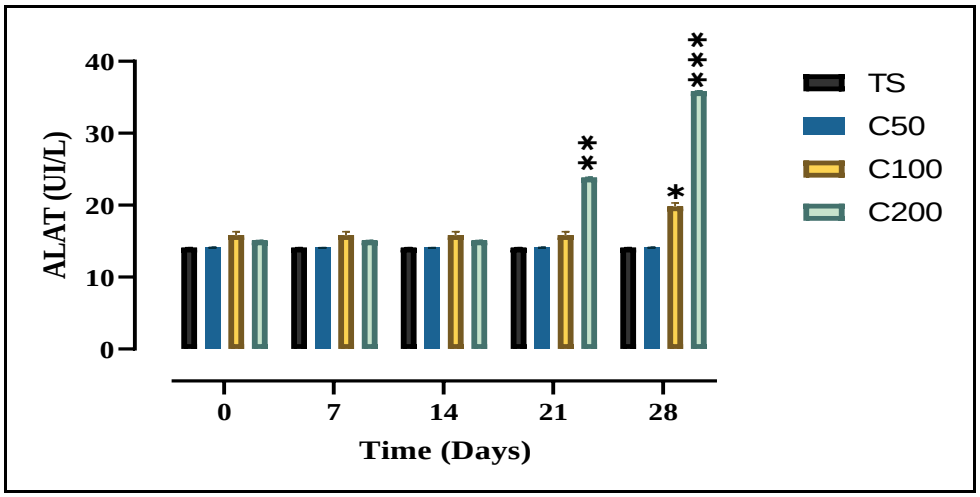

Figure 7: Changes in serum alanine aminotransferase (ALAT) levels during 28 days of treatment of healthy rats with Creatinor

Values are expressed as mean, followed by standard error of the mean (M ± SEM)

*n = 6; * p < 0.05; ** p < 0.01, *** p < 0.001 compared to controls*

TS: Control rats, C50: Rats treated with Creatinor at 50 mg/kg bw., C100: Rats treated with Creatinor at 100 mg/kg bw, C200: Rats treated with Creatinor at 200 mg/kg bw

Dose-response effects of Creatinor on the histological structures of the liver and kidney of healthy rats after 28 days of treatment

Effects of Creatinor on the histological structure of the kidney of healthy rats

Figure 8 shows histological sections of the kidney of healthy rats and rats treated for 28 days with 50, 100 and 200 mg/kg bw of Creatinor. Histological sections of healthy rat kidney show renal tissue with nephrons with glomeruli, glomerular chambers and distal convoluted tubules (Figure 8 A). Histological sections of the kidney of rats given 50 mg/kg bw Creatinor showed

normal nephrons, with glomeruli, glomerular chambers and distal convoluted tubules similar to those of the control rat kidney (Figure 8 B). Histological sections of rat kidneys treated for 28 days with Creatinor at 100 mg/kg bw showed fewer nephrons, with poorly condensed glomeruli and partially shrunken glomerular chambers, compared with those of control rat kidneys (Figure 8 C). Histological sections of rat kidneys treated with Creatinor 200 mg/kg bw after 28 days of treatment showed several anatomical abnormalities. The kidneys showed narrowing of the glomerular chamber, condensation of the inner surface of the glomerulus with destruction of the capillary coves and necrosis of the nephrons. The renal structure is disorganized, with a lot of cellular debris (Figure 8 D).

Effects of Creatinor on the histological structure of liver in healthy rats

Figure 9 shows histological sections of the liver of healthy rats and rats treated daily for 28 days with Creatinor at doses of 50, 100 and 200 mg/kg bw. Observation of histological sections of the liver of healthy control rats shows a normal architecture of hepatic cells (Figure 9 A). Indeed, several types of hepatic cells are well differentiated, including tetraploid nuclei, sinusoidal cells, portal veins and hepatic arteries. When rats were treated for 28 days with Creatinor at doses of 50 mg/kg bw (Figure 9 B) and 100 mg/kg bw (Figure 9 C), histological sections of the liver of these animals showed histological structures similar to those of the liver of control rats, with tetraploid nuclei of the portal veins and normal hepatic arteries. In contrast, histological sections of rat liver treated with 200 mg/kg bw of Creatinor (Figure 9 D) show a superficial modification of the architecture of the hepatic cells, with flattened tetraploid nuclei and narrowed portal veins. Some hepatocytes appear necrotic.

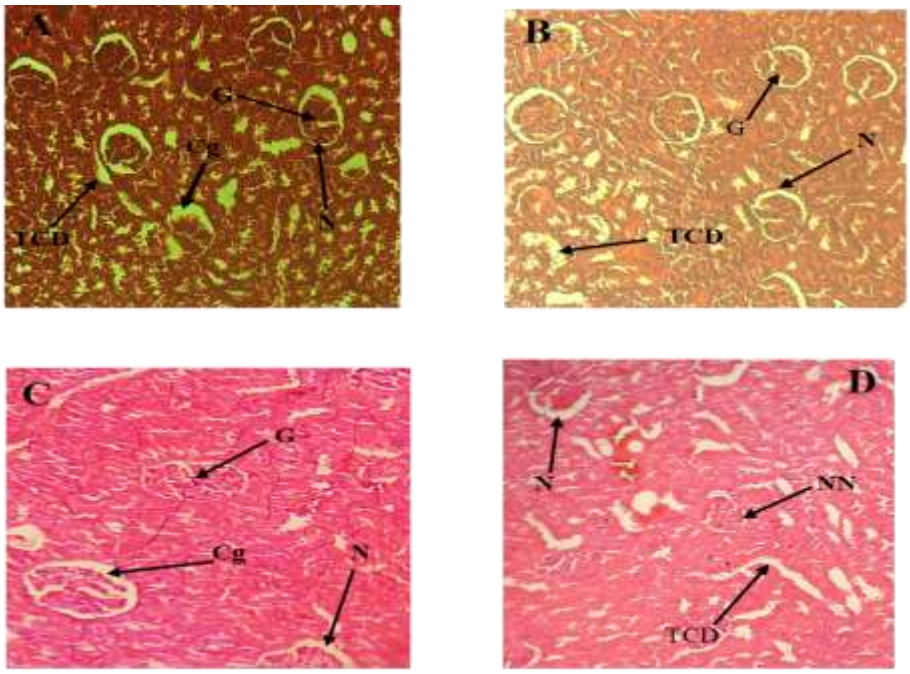

Figure 8: Effects of Creatinor on the histological structures of healthy rat kidneys after 28 days of treatment

A: Control rat kidney

B, C, and D: Kidneys of rats treated with Creatinor at 50 mg/kg bw (B), 100 mg/kg bw (C), and 200 mg/kg bw (D)

N: Nephron; G: Glomerulus; Cg: Glomerular chamber; TCD: Distal convoluted tubule; NN: Necrotic nucleus, Staining: Hematein-eosin; G x 100

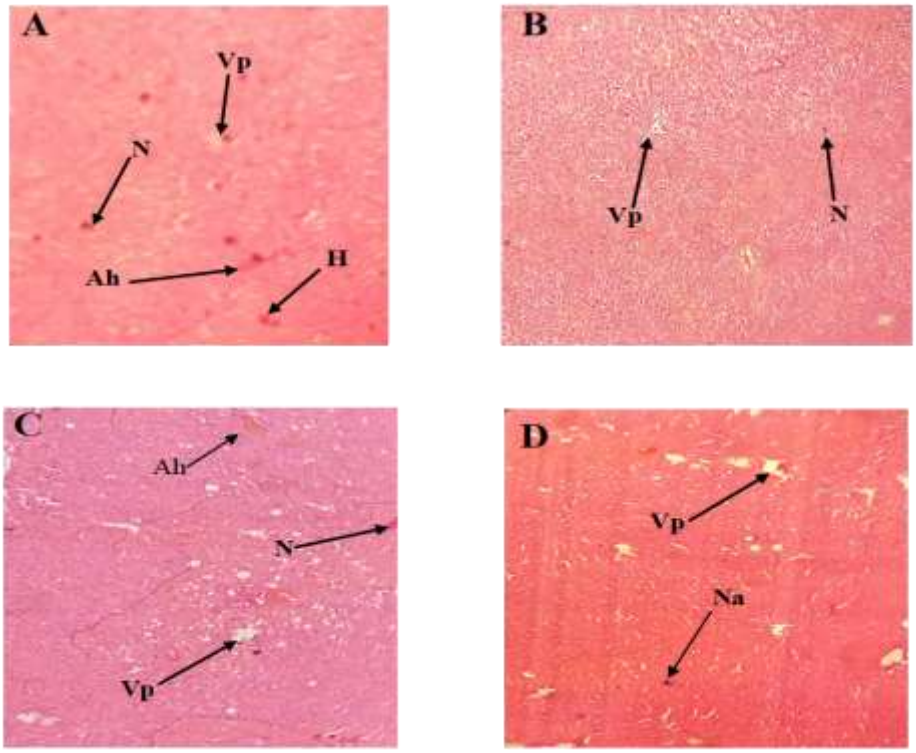

Figure 9: Effects of Creatinor on the histological structures of the liver of healthy rats after 28 days of treatment

A: Control rat liver

B, C and D: Livers of rats treated with Creatinor at 50 mg/kg bw (B), 100 mg/kg bw(C) and 200 mg/kg bw (D)

Ah: Hepatic artery; Vp: Portal vein; H: Hepatocyte; N: Nucleus

Staining: Hematein-eosin; G x 100

Discussion

A study of the acute toxicity of Creatinor in mice, conducted in accordance with OECD guideline 423 (5), showed that a single oral administration of this substance at doses of 5, 50, 300, 2000 and 5000 mg/kg bw did not result in symptomatic toxicity. Indeed, the behaviour and physiology of treated mice remained normal, identical to those of control mice. Furthermore,

Creatinor, administered to mice at doses up to 5000 mg/kg bw, did not cause any deaths in treated animals. These results indicate that Creatinor has an LD50 greater than 5000 mg/kg bw. Thus, this substance is non-toxic when administered orally, according to the globally harmonized classification system of the (5, 8, 9). Similar results were obtained by (10) who showed that the aqueous extract of Cassia occidentalis leaves (Caesalpiniaceae) did not induce death in mice given these substances by gavage at a dose of up to 5000 mg/kg bw. Consequently, these different substances are not toxic. Monitoring of the weight development of healthy rats during 28 days of experimentation showed a non-significant increase or loss of body mass in these animals treated with Creatinor at doses of 50 and 100 mg/kg bw respectively, compared with that of control rats. These non-significant effects of Creatinor at doses of 50 and 100 mg/kg bw on the weight growth of rats could be explained by a physiological adaptation of the animals to these doses of the substance (11) and indicate that it has no adverse effects on their body development. Similar results were also reported by (12,10) who respectively showed that aqueous extracts of *Chrysophyllum perpulchrum* (Sapotaceae) and Cassia occidentalis (Caesalpiniaceae) had no significant effects on the weight growth of healthy rats. However, Creatinor at a dose of 200 mg/kg bw caused a significant reduction (22.32%) in the body weight of rats. This suggests that this substance, at a dose of 200 mg/kg bw, could induce undesirable side effects. Changes in body weight are used as an indicator of the undesirable side effects of drugs, chemicals and bioactive substances (13).

Treated animals that survive should lose no more than 10% of their initial body mass (14). In general, body weight loss and internal organ weight gain in rats reflect toxicity following exposure to toxic substances (15). A significant reduction in the weight growth of rats treated with aqueous extracts of Ajuva iva (Lamiaceae) was also observed by (16).

Administration of Creatinor at doses of 50, 100 and 200 mg/kg bw for 28 days resulted in a dose-dependent decrease in the relative weights of the kidneys and liver of rats, with non-significant, insignificant and significant effects on these organs, respectively. These results suggest that Creatinor may have dose-dependent effects on renal and hepatic function. The liver, kidneys and heart are the main targets of these substances. Plant-based products, when ingested into the body over a period of time, can be toxic to important organs such as the kidneys, liver and heart, due to their various roles in the human body (17). Once damaged, these organs release their enzymatic or protein content into the bloodstream. Measurement of creatinine and urea as markers of impaired renal function (16) in healthy rats treated with Creatinor at doses of 100 and 200 mg/kg bw showed significant dose-dependent increases in their levels from day 21 of

Creatinor administration. For the 50 mg/kg bw dose administered, the values of these biochemical parameters (creatinine and urea) did not vary significantly over the 28 days of experimentation. Thus, this dose of Creatinor did not affect renal function. On the other hand, from the 21st day of administration, Creatinor at a dose of 100 mg/kg bw would cause renal dysfunction which would increase with increasing dose of this substance.

Creatinor could therefore cause renal tissue damage and nephrotoxic effects in rats when administered long-term at doses of 100 and 200 mg/kg bw, with a more pronounced toxic effect at 200 mg/kg bw. Creatinine is the major biological marker of renal impairment (18). Changes in serum creatinine levels are a reliable indicator of glomerular filtration disorders and renal dysfunction (19). Also, in cases of renal failure, serum urea concentration increases (20). Other studies have also shown that increases in serum creatinine and urea levels are generally linked to kidney damage or dysfunction, renal failure or even nephrotoxicity linked to excess uric acid in the blood (21, 22). The nephrotoxic effects of Creatinor at doses of 100 and 200 mg/kg bw are confirmed by its effects on the histological structures of kidneys from healthy rats treated with this substance. In healthy rats given Creatinor at 50 mg/kg bw for 28 days, the histological structures of the kidneys were not altered. However, at Creatinor 100 mg/kg bw there were some changes in the histological structure of the kidneys, in particular fewer nephrons, less condensed glomeruli and partially narrowed glomerular chambers. Very marked disorganization and necrosis of the renal tissue appeared at the Creatinor dose of 200 mg/kg bw, indicating more pronounced nephrotoxicity of the substance at this dose. These results differ from those of (23,24) who respectively showed that the ethanolic extract and aqueous extract of the leaves of Rauvolfia vomitoria (Apocynaceae) did not cause any significant changes in the structure of the kidneys of healthy rats poorly condensed glomeruli and partially narrowed glomerular chambers.

Measurement of transaminases (ASAT and ALAT) revealed the impact of different doses of Creatinor (50, 100 and 200 mg/kg bw) on the liver tissue of healthy rats. The results show that administration of 50 mg/kg bw of Creatinor did not cause any significant variation in ASAT and ALAT levels over the 28 days of experimentation, but these levels did not increase significantly until the 28th day of treatment with 100 mg/kg bw. of this substance. On the other hand, in rats treated with 200 mg/kg bw of Creatinor, levels of these liver enzymes increased significantly from the 21st day of treatment in healthy rats. These results suggest that Creatinor, when administered over a long period, becomes toxic for the liver, and this hepatotoxicity occurs more rapidly when the dose of this substance is 200 mg/kg P.C. In fact, transaminases are tissue enzymes present in the liver, but also in other tissues. The increase in ASAT and ALAT levels in

the blood is due to their release following damage to liver cells (15). Hepathotoxic effects were also observed by (25), who reported an increase in serum transaminase levels in rats receiving extracts of Argemone mexicanal (Papaveraceae) and Aphania senegalensis (Sapindaceae) respectively. Observation of histological sections of the livers of healthy rats after 28 days of treatment with Creatinor showed structural damage at certain doses, justifying the toxic effects of this substance on this vital organ. In fact, administration of Creatinor at doses of 50 and 100 mg/kg bw to healthy rats for 28 days did not alter the structure of the rats' livers. These results mean that these doses administered over a 28-day period had no adverse effects on liver tissue. On the other hand, with the 200 mg/kg bw dose of Creatinor administered to healthy rats for 28 days, there was a structural modification of the hepatic tissue, in particular with the necrosis of some hepatocytes. This result indicates that Creatinor, at this dose, is hepatotoxic. These results are similar to those obtained by (26) who showed the presence of necrotic hepatocytic cells on the liver of healthy rats treated with the aqueous extract of the roots of *Rauvolfia vomitoria* (Apocynaceae). The greater hepatic toxicity of Creatinor at 200 mg/kg bw justifies the adverse effects of this dose of the substance on weight growth and the greater reduction in the relative liver weight of treated rats, compared with that induced by the 100 mg/kg bw dose.

CONCLUSION

The study of Creatinor's acute toxicity shows that it is non-toxic when administered orally to mice. This oral route of administration would therefore be recommended for the therapeutic and pharmacological uses of this substance. Creatinor, administered orally over a period of 21 days or more to healthy rats at doses of 100 and 200 mg/kg bw, has dose-dependent effects on body mass and on the renal and hepatic tissues of treated rats. The 100 mg/kg bw dose did not affect body weight growth but had some effect on renal and hepatic tissues from day 21 of treatment, whereas the 200 mg/kg bw dose had adverse effects on body development and induced more marked and earlier hepatic and renal toxicity. In contrast, 50 mg/kg bw had no effect on any of these parameters. Thus, the use of Creatinor at doses higher than 50 mg/kg bw, in particular 100 mg/kg bw used by traditherapists in the treatment of renal failure, must be carried out with particular care when this substance is administered over a long period (greater than or equal to 21 days) in healthy animals, which could lead to adverse effects, in particular nephrotoxicity and hepatotoxicity.

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