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Oral toxicity of trypanocidal molecules hemi-synthesized in *Cymbopogon citratus* essential oil

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ABSTRACT

To ensure the safety of trypanocidal molecules of thiosemicarbazones hemi-synthesized in essential oil of *Cymbopogon citratus*, toxicology studies were conducted. These studies which were performed on rats "Wistar" had focused on our most active molecules: citral thiosemicarbazone, citral semicarbazone, citral 4-phenylthiosemicarbazone and the starting substrate which was the essential oil of *Cymbopogon citratus*. From these studies, it appears that citral semicarbazone, citral 4-phenylthiosemicarbazone and essential oil were no toxic as LD_{50} > 2000 mg/kg. Citral thiosemicarbazone was slightly more toxic with a LD_{50} = 315 mg/kg. The study of biochemical parameters revealed that the use of a high dose of the compounds may affect kidney function. A strong dose of essential oil of *Cymbopogon citratus* could affect liver function. It appears that the citral 4-phenylthiosemicarbazone is a very promising compound because it had an excellent antitrypanosomal activity and had very low toxicity (IC_{50} = 1.96 µM; LD_{50} > 2000 mg/kg).

Keywords: Trypanocidal thiosemicarbazones, toxicology, essential oil, *Cymbopogon citratus*, biochemical parameters.

INTRODUCTION

The African continent is unfortunately supported the birth and proliferation of many microorganisms against which we must fight every day. We were interested in this work to a group of trypanosome parasites (*Trypanosoma brucei brucei*), responsible for human and animal African trypanosomiasis (sleeping sickness) (Feasey et al., 2010; Boelaert et al., 2010). The drugs used for the treatment of this disease date back several decades. And so, it would be very interesting to develop other more effective and less toxic (Barrett et al., 2000).

The many properties of thiosemicarbazones have been demonstrated in the literature (Kolocouris et al., 2002; de Oliveira et al., 2008; Fatondji et al., 2010; Glinma et al., 2011). In our previous work, thiosemicarbazones were hemi-synthesized *in-situ* in the essential oil of *Cymbopogon citratus* (Sakirigui et al., 2011, 2012). The

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antiparasitic properties on *Trypanosoma brucei brucei* of these molecules were also studied (Sakirigui et al., 2011, 2012). It appears that the citral thiosemicarbazone and citral 4-phenylthiosemicarbazone had a very interesting trypanocidal activity against these parasites. Furthermore, the toxicity test on *Artemia salina* tells us that citral 4-phenylthiosemicarbazone is moderately toxic. According to the work of Santos et al. (2003), this product could therefore have anti-tumor properties.

However, upon discovery of any substance for therapeutic power, the acute toxicity tests are a preliminary step in preclinical experiments that are a prerequisite for any test in humans. Thus, the objective of this work was therefore to determine the acute toxicity of citral semicarbazone, citral thiosemicarbazone, citral 4-phenyl thiosemicarbazone and acute toxicity of the essential oil of *Cymbopogon citratus*, oil in which thiosemicarbazones molecules had been semi-synthesized. The experiments will be performed on rats "Wistar", laboratory animal chosen for toxicological tests in order to assess the minimum lethal dose, the lethal dose 50 (LD_{50}) and the no-observed-adverse-effect level (NOAEL), by proceeding by Ahmed et al. (2010) methods. *In-vivo* effects of these molecules will also be determined by following the evolution of some biochemical parameters.

MATERIAL AND METHODS

The study of toxicity was performed on adult male and female rats, type "Wistar". The experimental protocol was carried out in accordance with OECD (Organization for Economic Cooperation and Development) guidelines. The animals were distributed in cages in the pet store. Animals had free access to water and food (standard diet). Rats are marked to permit individual identification and kept in their cages to acclimate them to living conditions for least five days before the experiment. All animals were weighed before the experiment, OECD (2000, 2001).

Determination of biochemical parameters

Administration of a single dose of 500 mg/kg of various compounds gives an idea about doses to prepare in order to find the Lethal Dose 50 (LD50) that could kill 50% of the treated animals. Biochemical parameters such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatinine, bilirubin, alkaline phosphatase (ALP) and total protein were measured in the blood of animals before and after administration compounds. In fact, these enzymes were used to assess liver and kidney function (Leise et al., 2014; Liu et al., 2008).

6 batches of 5 rats whose weight varies between 120 and 198 g were made and distributed in 6 cages. These were fasted 14 hours before the experiment (from 6 o'clock pm to 8 o'clock am) to allow proper assimilation of the product. A single dose of 500 mg/kg of citral semicarbazone, citral thiosemicarbazone and citral 4-phenyl thiosemicarbazone was prepared in corn oil where they remained in perfect suspension. A dose of 500 mg/kg of *Cymbopogon citratus* essential oil from which the compounds were made was also administered to another group of rats. The blood of each rat was taken in a test tube before and 24 h after administration of the products. The samples contained in test tubes were put in the cold for thirty minutes and then centrifuged for obtaining serum from what will be dosed different enzymes (Balcombe et al., 2004; Castelhano-Carlos et al., 2009; OECD 2000, 2001).

The rats were anesthetized in a jar containing cotton soaked in ether. Using a capillary tube blood is drawn next to the eye. It was collected in a test tube and then placed in the refrigerator for 15 to 30 minutes. After 15 minutes of centrifugation, the serum was collected using a micropipette and then packaged in a tube. This serum was used for determination of biochemical parameters.

Determination of lethal doses

For the lethal dose of a substance determination, the OECD recommended to administrate increasing doses of this substance to animals in several groups. Doses administered should be between 5 mg/kg and 5 g/kg body weight to help locate the substances tested on chemical toxicity of the comparative scale of OECD (2000, 2001).

6 lots of 3 rats were given repeated doses of 100 mg/kg, 250 mg/kg, 500 mg/kg, 750 mg / kg, 1000 mg/kg and 2000 mg/kg of citral semicarbazone. The second group consisted of 4 groups of 3 rats to which successive doses were administered 100 mg/kg, 250 mg/kg, 500 mg/kg, 750 mg/kg of citral thiosemicarbazone. In the case of citral 4-phenyl thiosemicarbazone, doses of 250 mg/kg, 500 mg/kg, 750 mg/kg, 1000 mg/kg and 2000 mg/kg were administered to 5 lots of 3 rats. The *Cymbopogon citratus* essential oil prepared at increasing doses of 250 mg/ml, 500 mg/ml, 750 mg/ml, 1000 mg/ml and 2000 mg/ml was also administered to 5 sets of 3 rats.

The control group consists of three rats which received corn oil. Indeed this oil contains little of substance that can influence the results.

After administration of different substances, the rats were observed for 14 days. The observation of the animals was based on parameters such as the variation in the weight of the animal, the external appearance; behavior change; behavioral responses to external stimuli (eg. noise, variation in light intensity), aggressiveness, food intake, impaired balance etc. Statistical analysis was performed using the STATISTICA software (version 4.1, Statsoft, Paris, France). Multiple comparisons were performed using ANOVA. The differences are considered significant at $p < 0.05$.

RESULTS

Lethal Doses determination

Citral semicarbazone

Average weight evolution during fourteen days of the animals before and after administration of different doses of citral semicarbazone were mentioned in Table 1. The weights of the rats which consumed a low dose of semicarbazone increased from the first to the fifteenth day. The rats which consumed a high dose of the product decreased in the first three days. No deaths were recorded with this product.

Citral thiosemicarbazone

All rats in this batch had experienced a decrease in weight. Apart from this aspect, the rats given a low dose showed signs of aggression (Table 2). Deaths were also recorded from a dose of 250 mg/kg after the day of experiment.

In the case of this compound, it was possible to determine the lethal dose from the curve reflecting the percentage of death compared to the dose received. The lethal dose is 315 mg/kg (Figure 1).

Citral 4-Phenyl thiosemicarbazone

A decrease in body weight of rats in this batch was observed the thirst three days. No significant weakness was felt in these rats. Only those who received the dose of 2000 mg/kg seemed tired and lost considerable weight (Table 3). Unlike thiosemicarbazone, no deaths were recorded with this product during the experiment. It is therefore less toxic than this one.

Oil of *C. citratus*

No significant reaction was observed in rats which had taken a dose of (500 mg/kg). They had a normal behavior of the next day 14. Decreased weight in these animals is observed from the dose of 1000 mg/kg (Table 4). Rats that received a high dose of the essential were in general, less agitated than the others.

Control rats

Corn oil had no effect on the control rats. A normal development of these rats was observed during the experiment.

Biochemical tests

Alanine aminotransferase (ALAT)

Alanine aminotransferase (ALAT) rate of different substances were indicated in Figure 2. Its levels which were lower, increased after administration of the products. This increase was significant in the case of the essential oil $p < 0.05$. Citral semicarbazone, unlike all products, had decreased the level of this enzyme.

Aspartate aminotransferase (ASAT)

Figure 3 indicated Aspartate aminotransferase (ASAT) rate of different substances. Essential oil also showed the high rate of ASAT. The increase of Aspartate aminotransferase was again significant in the

case of the essential oil. Citral semicarbazone further inhibited the production of that enzyme.

Alkaline phosphatase (ALP)

The increase in Alkaline phosphatase was significant in all cases, but it was very pronounced with citral semicarbazone and citral 4-phenylthiosemicarbazone (Figure 4).

Bilirubin

The increase in bilirubin levels was significant in the case of the essential oil and citral semicarbazone (Figure 5). This increase was moderate in the other cases.

Creatinine

Increased creatinine levels were elevated in rats that consumed citralthiosemicarbazone and citral 4-phenylthiosemicarbazone (Figure 6). No increase was observed with citralsemicarbazone and essential oil.

Total protein

Total protein high rate was watched in witness rats (Figure 7).

Table 1: Average weight of rats after administration different doses of citral semicarbazone.

Compound		Average weight (g)							
	Dose (mg/kg)	D0	D1	D 3	D 5	D 7	D 9	D 11	D 14
Citral semicarbazone	250	165 ± 24	169 ± 22	176 ± 21	182 ± 18	185 ± 14	187 ± 13	188 ± 14	181 ± 3
	500	136 ± 15	134 ± 15	138 ± 18	145 ± 17	151 ± 17	157 ± 18	158 ± 15	156 ± 16
	750	137 ± 9	140 ± 11	147 ± 10	156 ± 10	163 ± 12	167 ± 12	167 ± 10	172 ± 11
	1000	115 ± 13	102 ± 16	98 ± 16	107 ± 16	114 ± 14	122 ± 13	125 ± 14	130 ± 13
	2000	116 ± 10	99 ± 12	101 ± 13	109 ± 12	116 ± 10	122 ± 7	127 ± 5	131 ± 5

D = Day

Table 2: Average weight of rats after administration different doses of citral thiosemicarbazone.

			Weight (g)							
	Dose (mg/kg)	**Marking**	**D0**	**D1**	**D3**	**D5**	**D7**	**D9**	**D11**	**D14**
Citral thiosemicarbazone	100	1	186	178	169	169	174	175	172	177
		2	168	150	155	148	154	158	161	168
		3	137	129	124	118	125	132	137	141
	250	1	184	175	171	174	179	177	175	178
		2	186	-	-	-	-	-	-	-
		3	156	147	145	151	158	161	164	169
	500	1	129	117	-	-	-	-	-	-
		2	137	-	-	-	-	-	-	-
		3	145	132	-	-	-	-	-	-
	750	1	133	-	-	-	-	-	-	-
		2	133	-	-	-	-	-	-	-
		3	138	-	-	-	-	-	-	-

D = Day - = Rat death.

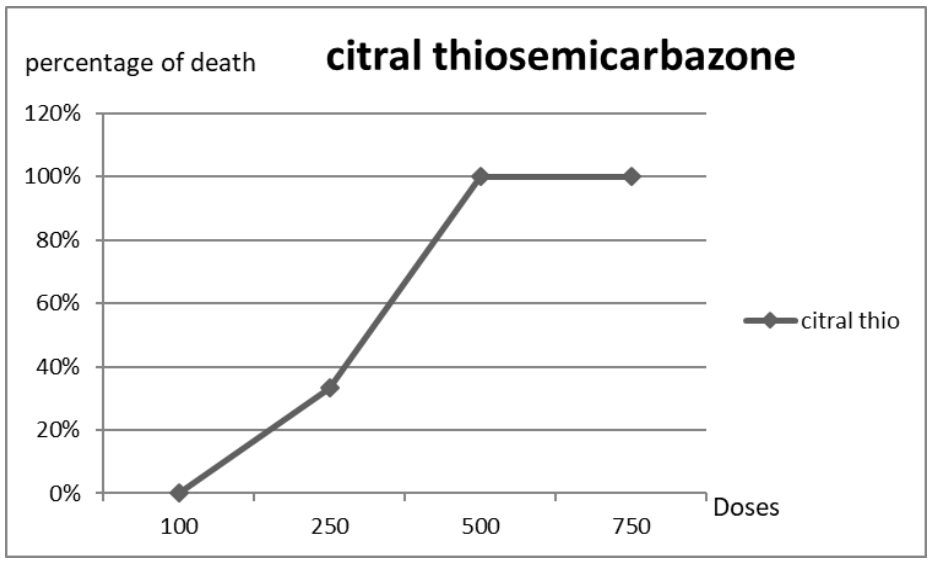


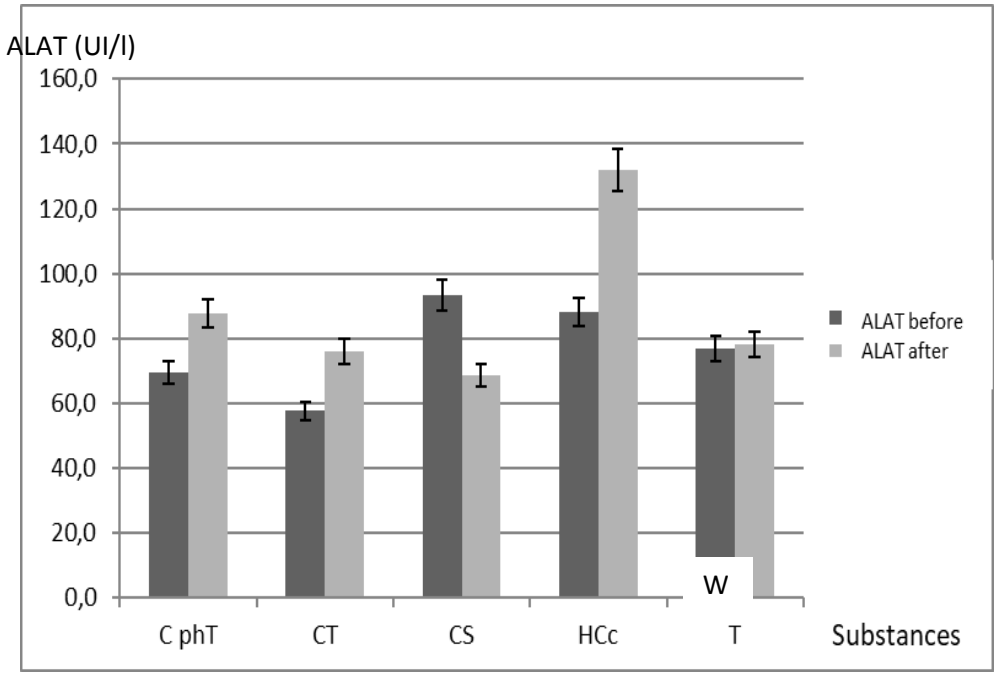
Figure 1: Percentage of deaths compared to the dose of citral thiosemcarbazone.

Table 3: Average weight of rats after administration different doses of citral 4-phenyl thiosemicarbazone.

		Average weight (g)							
Compound	**Dose (mg/kg)**	**D 0**	**D 1**	**D 3**	**D 5**	**D 7**	**D 9**	**D 11**	**D14**
Citral Phényl thiosemicarbazone	250	175 ± 11	166 ± 9	174 ± 8	182 ± 10	189 ± 8	194 ± 6	192 ± 8	198 ± 9
	500	145 ± 8	144 ± 11	142 ± 9	148 ± 9	156 ± 10	162 ± 10	165 ± 5	171 ± 4
	750	142 ± 5	131 ± 4	138 ± 7	145 ± 8	152 ± 7	157 ± 7	164 ± 8	170 ± 9
	1000	135 ±	129 ±	132 ±	138 ±	147 ±	152 ±	155 ±	164 ±
	2000	124 ± 10	96 ± 11	101 ± 10	107 ± 9	115 ±8	123 ± 8	129 ± 4	137 ± 1

Table 4: Average weight of rats after administration different doses of *C. citratus* essential oil.

		Average weight (g)							
Substance	**Dose (mg/kg)**	**D 0**	**D 1**	**D 3**	**D 5**	**D 7**	**D 9**	**D 11**	**D14**
Essential oil	250	180 ± 5	178 ± 8	164 ± 8	182 ± 8	179 ± 6	164 ± 9	192 ± 8	198 ± 9
	500	151 ± 9	153 ± 10	143 ± 7	148 ± 9	156 ±12	152 ± 10	165 ± 5	171 ± 4
	750	139 ± 8	145 ± 5	148 ± 7	145 ± 8	152 ± 5	157 ± 7	164 ± 8	170 ± 9
	1000	142 ±8	131 ±17	139 ±6	138 ±	147 ±5	152 ±12	179 ±15	156 ±17
	2000	165 ± 6	120 ± 11	111 ± 9	107 ± 9	115 ±18	123 ± 8	149 ±14	139 ± 8



Cpht = citral 4-phenyl thiosemicarbazone; CT = citral thiosemicarbazone; CS = citral semicarbazone; HCc = *C. citrates* essential oil; W = Witnesses.

Figure 2: ALAT rate before and after administration of substances.

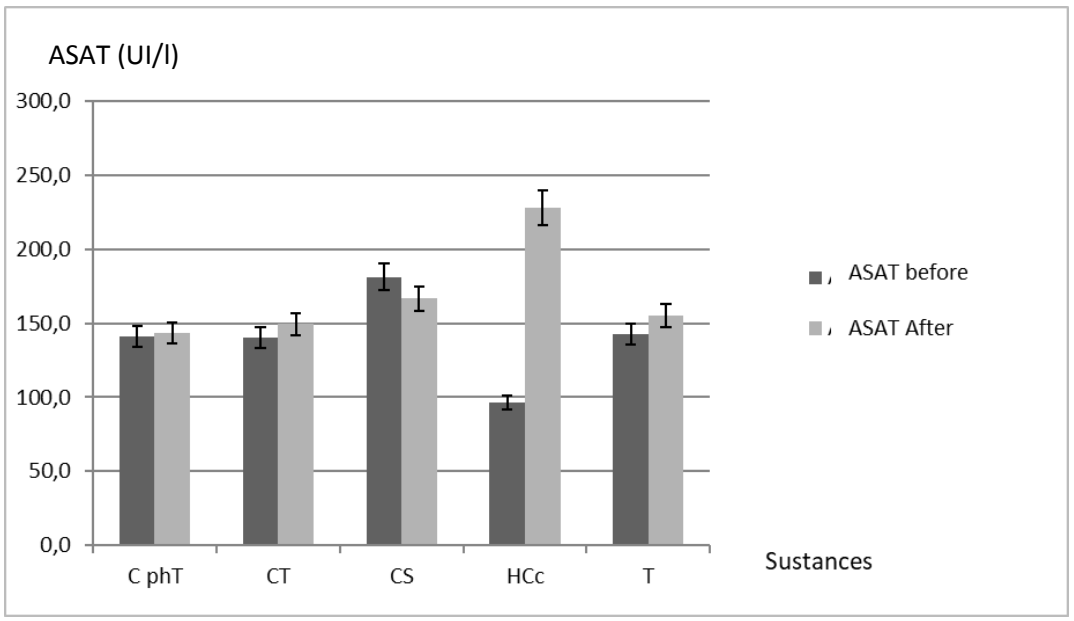


Figure 3: ASAT rate before and after administration of substances.

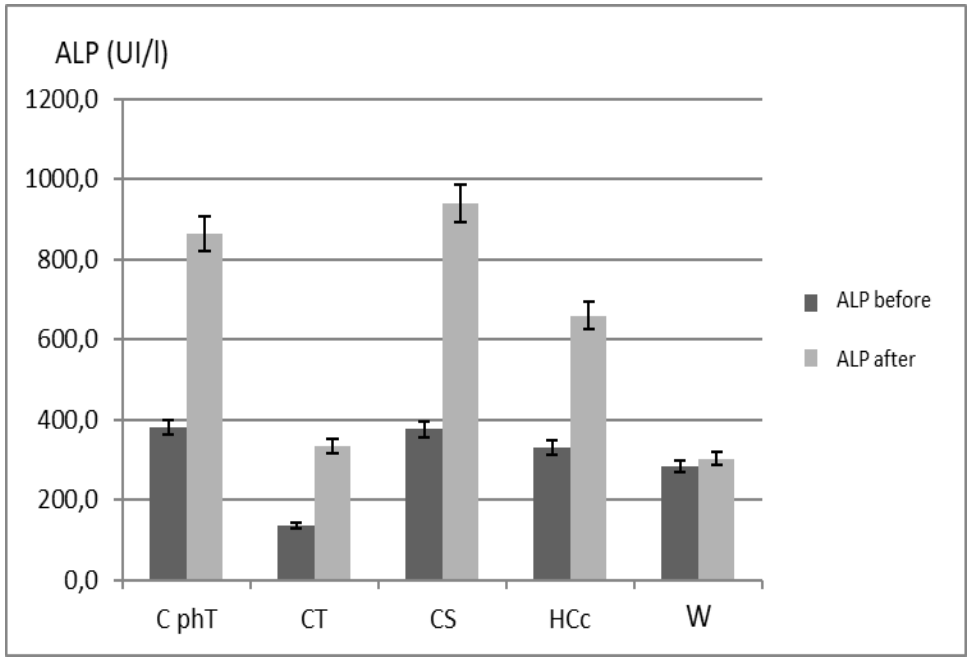
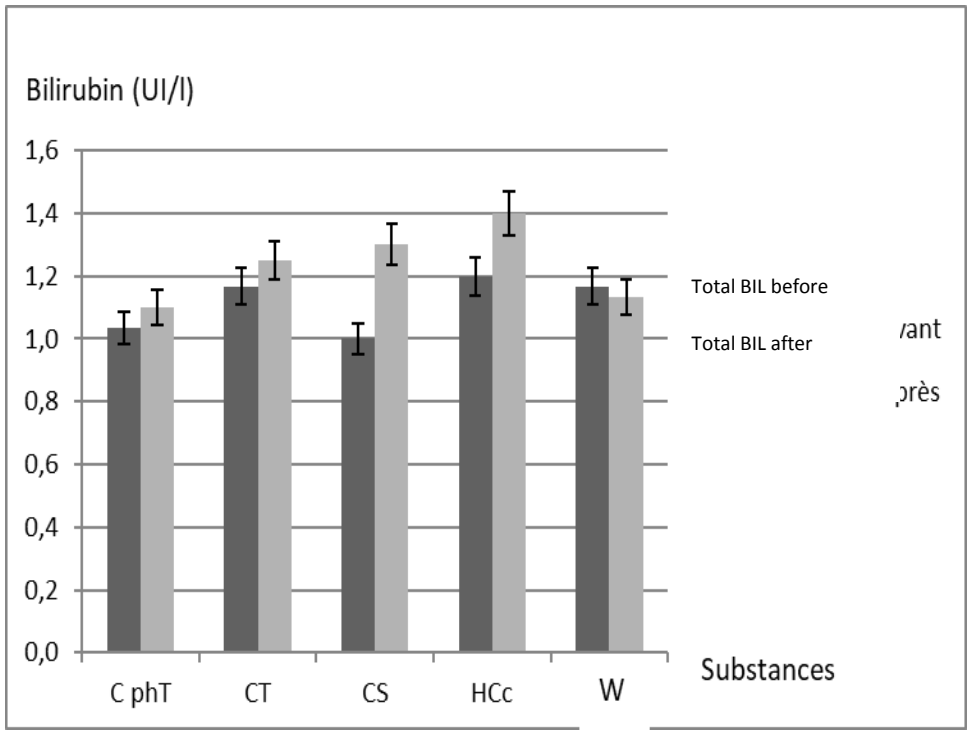
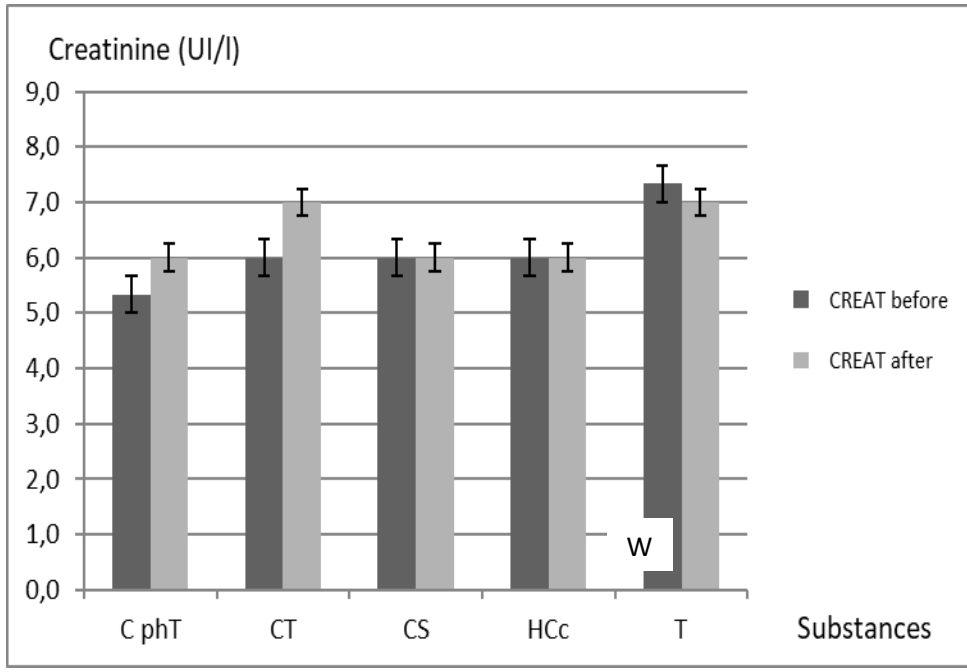


Figure 4: ALP rate before and after administration of substances.



BIL = Bilirubine

Figure 5: bilirubin ratios before and after administration of substances.



CREAT = Creatinine.

Figure 6: creatinine levels before and after administration of substances.

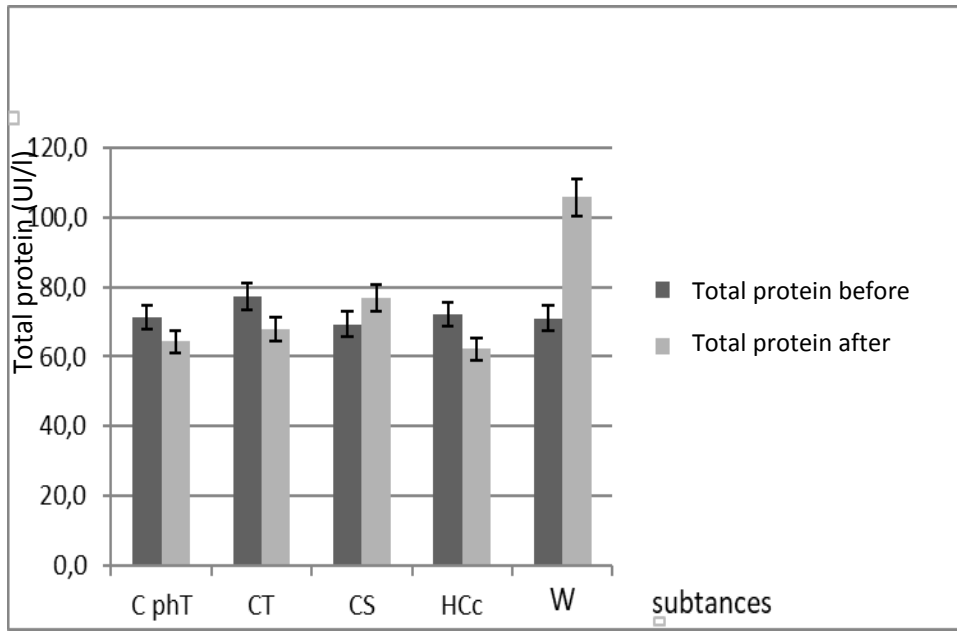


Figure 7: Protein ratios before and after administration of substances.

Table 5: Weight evolution of animals before and after administration of corn oil.

Lot	J0	J1	J3	J5	J7	J9	J11	J14
Witnesses	128 ± 7	129 ± 8	134 ± 8	138 ± 7	144 ± 6	149 ± 6	155 ± 4	159 ± 6

DISCUSSION

Lethal Doses determination

Citral semicarbazone, Citral thiosemicarbazone and Citral 4-phenylthiosemicarbazone were obtained by hemi-synthesis in the essential oil of *Cymbopogon citratus*. Citral thiosemicarbazone (IC_{50} = 7.61 µM) Citral 4-phenylthiosemicarbazone (IC_{50} = 1.96 µM) showed a good activity on *Trypanosoma brucei brucei* in ours previous work (Sakirigui et al., 2011, 2012). Citral semicarbazone's activity was low (IC_{50} = 234.64 µM).

No death was registered in rats who consumed various doses of citral semicarbazone, citral 4-phenylthiosemicarbazone and essential oil during the 14 days that have long these experiences. No particular behavior was observed in rats at doses < 1000 mg/kg of these substances. Unlike other cases, they had also made weight gain the next day after the products administration (Tables 1, 2). A weakness was observed in rats which took the dose of 2000 mg/kg of citral semicarbazone (Table 1).

After citral 4-phenylthiosemicarbazone administration, weight gain two days after the experiment was observed in rats. The lethal dose of this compound is greater than 2000 mg/kg. According to the toxicity scale, this compound is no toxic. This compound is very interesting because its very pronounced trypanocidal activity had been demonstrated in our previous work (Sakirigui et al., 2011; 2012).

Cymbopogon citratus essential oil, the natural essence from which are hemi-synthesized the compounds, indicate no death for the doses ≤ 2000mg/kg. Its trypanocidal activity was also good (6.80 µg/mL). The lethal dose of this substance is also greater than 2000 mg/kg. So it is no toxic too. But the dissection of the animals which took the rate of 2000 mg/kg after 14 days showed a burning of their intestines in the case of essential oil and the contrary in the molecule case. This reaction is certainly due to the presence of citral (aldehyde which irritates mucous membrane in many cases) in large quantities in the oil (Andrade et al., 2009). This work brings more to the many works that have dismantled the pharmacological properties of this oil (Negrelle et al., 2007; Degnon et al., 2016; Alitonou et al., 2012; Diop et al., 2017).

It was possible to determine the lethal dose in the case of citral thiosemicarbazone from the curve reflecting the percentage of death compared to the dose received. The lethal dose is 315 mg/kg (Figure 1). Minimum Lethal Dose of this compound was also determined. It is the dose that kills a minimum of the animals treated after administration of product. Its value is 250 mg/kg. The dose of 100 mg/kg had no significant effect in rats during 14 days of experiment (Table 2). It is therefore the no-observed-adverse-effect level (NOAEL) (Ahmed et al., 2010). According to the toxicity scale, this product as a pure compound is moderately toxic. It can be used in small doses against parasitic diseases account held its very high trypanocidal activity (Santos Pimenta et al., 2003; Sakirigui et al., 2011).

According OCDE (2000, 2001) guidelines, citral semicarbazone, citral 4-phenylthiosemicarbazone and *C. citratrus* essential oil were no toxic. Citral thiosemicarbazone was slightly more toxic.

Biochemical tests

Tests on the biochemistry and liver function measure various enzymes as liver released into the blood, as well as other liver function. Elevation of liver enzymes may

occur when the liver sustains are damaged (Hamilton et al., 2016; Leise et al., 2014; Kew et al., 2000).

Alanine aminotransferase (ALAT), is an enzyme produced by the liver cells (hepatocytes). The ALAT level in the blood increases when hepatocytes are damaged or destroyed at a faster rate than normal (Hamilton et al., 2016; Leise et al., 2014; Kew et al., 2000). Drugs, alcohol, toxins, viruses and other substances cause damage to liver cells that may contribute to the elevation of ALAT. The death of liver cells also causes an increase in ALAT levels. ALAT levels are often used to assess the degree of inflammation and liver damage at any point in the progression of the disease. It would therefore not interest that a product used in the treatment of a disease causing its disproportionately increase. No significant increase in the levels of this enzyme is observed in the rats to which were administered synthetic products ($P > 0.05$) (Figure 3).

The only significant increase ($P < 0.05$) was observed in the rats that consuming the essential oil of *Cymbopogon citratus*. This rate remained invariant in control rats that consumed corn oil. The rates of animals that consumed the citral semicarbazone decreased after 24 h.

Aspartate aminotransferase (ASAT) is an enzyme similar to ALAT but is not as specific for liver disease. In many cases of liver inflammation, ALAT and ASAT levels are high. The above observations are substantially the same with respect to the change in ASAT levels in the blood serum.

The variation is very significant ($P < 0.05$) in rats consuming the essential oil. This variation is negligible in other cases (OCDE 2000, 2001).

Phosphatase alkaline (ALP) is an enzyme produced in the bile ducts and bones, and it is found in the liver. Level increases in the presence of hepatitis, cirrhosis and other diseases. Some medications can also increase its level.

In the case of this work, there was a significant increase in ALP rate in animals that consumed the semicarbazone citral, citral 4-phenyl thiosemicarbazone, the increase is moderate in rats that consumed the thiosemicarbazone citral and essential oil. No difference in the rate is observed in control rats (Figure 3).This increase is not very worrisome insofar some drugs produce the same effect (Kew et al., 2000).

Bilirubin is the main product of the degradation of old red blood cells. Red blood cells release of hemoglobin, "heme" portion is subsequently decomposed into bilirubin. When liver function is impaired, as in the case of acute hepatitis or the final stage of liver disease, bilirubin accumulates in the blood and causes yellowing of the skin and eyes, this condition is called jaundice. No significant difference was observed 24 h after administration of the products. The variations were all moderate. The increase in this rate was all time high in rats consuming the semicarbazone citral ($P < 0.05$) (Figure 4).

In general, there is a small variation in enzyme levels characterizing liver functions in rats which consumed 500 mg/kg of various synthetic compounds. Changes in these enzymes in rats which consumed 500 mg/kg of essential oil remain significant. *Cymbopogon citratus* essential oil, substrate from which the compounds were hemi-synthesized could damage liver function than compounds. From these analyzes, it is clear that, the different products do not significantly affect liver functions provided.

Renal function measured by laboratory tests that include blood urea nitrogen, creatinine and uric acid. In this work, only creatinine levels will be measured. The content of creatinine in blood is the most common measure of kidney function. After administration of the products, Kidneys plaid a vital role in the elimination of organic waste and in regulating blood pressure. Any imbalance in kidney function could be life-threatening (Sirwal et al., 2004).

The essential oil as citral semicarbazone, had no effect on creatinine levels in blood serum. Citral thiosemicarbazone and citral 4-phenyl thiosemicarbazone had caused a slight

increase in levels of this enzyme ($P > 0.05$) (Figure 7).

In general, the rates of liver enzymes assayed in the blood serum of control rats are similar before and after administration of corn oil (solvent used in this work). So we could say that this oil had no effect on the organs that produce different enzymes. The exceptional case of proteins would certainly due to the absence of liver function disturbance (Figure 8).

The AST, ALT and PAL were significantly elevated ($P > 0.05$) in rats which consumed a dose of 500 mg/kg of essential oil of *Cymbopogon citratus*. The change in creatinine level was greater with the pure compounds. In high doses, thiosemicarbazones compounds may be affect the kidneys and essential oil may be assigned the liver.

Conclusion

Any substances, either natural or synthetic may become dangerous beyond a given dose. That was the case of the essential oil of *Cymbopogon citratus* and thiosemicarbazones compounds. Citral semicarbazone, Citral 4-phenylthiosemicarbazone essential oil of *Cymbopogon citratus* are no toxic. Citral thiosemicarbazone is slightly more toxic. In high doses, the essential oil may affect the liver function. But it has no significant effect on kidney function. Unlike oil, in high dose, synthetic compounds may affect kidney function. The liver function is virtually unaffected by them.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The authors have participated in various ways in the design of this article. AS is the principal investigator of the work. RHF and EYL provided technical support and read the manuscript. The various works were carried out in the laboratories led by Professors AA and JDG, who also supervised the work.

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REFERENCES

Alitonou GA, Avlessi F, Tchobo F, Noudogbessi JP, Tonouhewa A, Yehouenou B, Menut C, Sohounhloue DK. 2012. Chemical composition and biological activities of essential oils from the leaves of *Cymbopogon giganteus* Chiov. and *Cymbopogon schoenanthus* (L.) Spreng (Poaceae) from Benin. *Int. J. Biol. Chem. Sci.*, **6**(4): 1819-1827. DOI: http://dx.doi.org/10.4314/ijbcs.v6i4.37

Ahmed FZB, Merzouk H, Bouanane S, Benkalfat NB, Merzouk SA, Mulengi JK, Narce M. 2010. Évaluation de la toxicité aiguë de la 2-hydroxy-méthyl-1 (N-phtaloyltryptophyl) aziridine chez le rat Wistar. *Ann. Toxicol Anal*., **22**(3): 115-121. DOI: 10.1051 / ata / 2010017

Balcombe JP, Barnard ND, Sandusky C. 2004. Laboratoire routine cause animal stress. *Comtemporary topics in laboratory animal science*, **43**(6): 42-51.

Boelaert M, Meheus F, Robays J, Lutumba P. 2010. Socio-economic aspects of neglected diseases: sleeping sickness and visceral leishmaniasis. *Ann. Trop. Med. Parasitol.*, **104**(7): 535-542. DOI: 10.1179/136485910X12786389891641

Castelhano-Carlos MJ, Baumans V. 2009. The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory animals*, **43**(4): 311-27.

Degnon GR, Adjou ES, Metome G, Dahouenon-Ahoussi E. 2016. Efficacité des huiles essentielles de *Cymbopogon citratus* et de *Mentha piperita* dans la stabilisation du lait frais de vache au

Sud du Bénin. *Int. J. Biol. Chem. Sci.*, **10**(4): 1894-1902. DOI: http://dx.doi.org/10.4314/ijbcs.v10i4.37

De Oliveira RB, de Souza-Fagundes EM, Soares RPP, Andrade AA, Kretti AU, Zani CL. 2008. Synthesis and antimalarial activity of semicarbazone and thiosemicarbazone derivatives. *Eur. J. Med. Chem.,* **43**: 183-188. DOI: 10.1016 / j.ejmech.2007.11.012

Feasey N, Wansbrough-Jones M, Mabey DCW, Solomon AW. 2010. Neglected tropical diseases. *British Medical Bulletin*, **93**: 179–200.

Glinma B, Kpoviessi SDS, Fatondji RH, Gbaguidi FA, Kapanda CN, Bero J, Lambert DM, Hannaert V, Quetin-Leclercq J, Moudachirou M, Poupaert J, Accrombessi GC. 2011. Synthesis, characterization and anti-trypanosomal activity of R-(-)carvone and arylketones-thiosemi carbazones and toxicity against *Artemia salina* Leach. *JAPS.,* **1**(8): 65-70.

Kew MC. 2000. Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet*., **355**: 591–592.

Kolocouris A, Dimas K, Pannecuoque C, Witvrouw M, Foscolos GB, Stamatiou G, Fytas G, Zoidis G, Kolocouris N, Andrei G, Snoeck R, De Clercq E. 2002. New 2-(1-adamantylcarbonyl) pyridine and 1-acetyladamantane thiosemicarbazones– thiocarbonohydrazones: cell growth inhibitory, antiviral and antimicrobial activity evaluation. *Bioorg. Med. Chem. Lett.*, **12**: 723-727.

Leise MD, Poterucha JJ, Talwalkar JA. 2014. Drug-induced liver injury**.** *Eur. J. Intern. Med*., **89**(1): 95-106.

Liu L. 2008. Expression, purification, and initial characterization of human alanine aminotransferase (ALT) isoenzyme 1 and 2 in High-five insect cells. *Protein Expr Purif.,* **60**(2): 225-231.

Negrelle RRB, Gomes EC. 2007. *Cymbopogon citratus* (DC.) Stapf: Chemical composition and biological activities. *Rev. Bras. Pl. Med.,* **9**: 80-92.

OCDE. 2000. Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Environmental Health and Safety Monograph Series on Testing and Assessment 19.

OCDE. 2001: Guidance document on Acute Oral toxicity. Environmental Health and Safety Monograph Series on testing and assessment. OCDE.

Sakirigui A, Kossouoh C, Gbaguidi F, Kpoviessi S, Fatondji RH, Poupaert J, Accrombessi GC. 2011. Hémi-synthèse et activités antiparasitaires sur *Trypanosoma brucei brucei* de thiosemicarbazones du citral dans l'huile essentielle de *Cymbopogon citratus* du Bénin. *J. Soc. Ouest-Afr. Chim.,* **31**: 11 - 20.

Santos Pimenta LP, Pinto GB, Takahashi JA, Silva LGF. 2003. Biological screening of Annonaceous Brazilian Medicinal Plants using *Artemia salina* (Brine Shrimp Test). *Phytomedicine,* **10**: 209-212.

Sirwal IA, Banday KA, Reshi AR, Bhat MA, Wani MM. 2004. Estimation of Glomerular Filteration Rate (GFR). *JK. Science*, **6**: 121–123.