



A New Heterocyclic 1H-perimidine synthesized 2-(2,3-dihydro-1H-périmidin-2yl)-6-methoxyphenol: Evaluation of Acute Toxicity in Wistar Rat

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Authors' contributions

This work was carried out in collaboration among all authors. Author TNT synthesized and characterized the new heterocyclic and designed the study. Author PKN co-designed the study, did the literature search, performed the statistical analysis and wrote the first draft of the manuscript. Author BD designed the experimental protocol of new chemical synthesized and authors KNJB, KCG, YOB and ZN gave facilities for the new product synthesis and helped in drafting final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: The 1H-perimidine, as novel source carbene ligand, is well known for its anti-fungal, anti-microbial or anti-tumor activities. Here, we aimed to study the acute toxicity in Wistar rat of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol, a new heterocyclic 1H-perimidine synthesized in our laboratory.

Materials and Methods: Five groups of males Wistar rats were intraperitoneally injected with 7 mg/kg, 40 mg/kg, 90 mg/kg, 130 mg/kg and 150 mg/kg dissolved in dimethyl sulfoxide (DMSO), and followed during 14 days. We noted any clinical signs of acute toxicity as body weight loss, salivation, tremor, convulsion among others, as well as food consumption and water intake level.

Results: The DL₅₀ of the new 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol was estimated to 65 mg/kg. The No Observed Adverse Effect Level (NOAEL) dose was 7 mg/kg because it did not caused mortality, clinical signs of acute toxicity, and not affected feed and water intake behavior. However, significant abnormalities as inflammation and necrosis were observed at doses-effects dependent in liver, when compared to NOAEL dose and vehicle.

Conclusion: The new heterocyclic 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol is considered as high toxicity grade product. From NOAEL dose, subsequent biological, toxicological, pharmacological and neurobehavioral studies are needed before using for clinical trials.

Keywords: Acute toxicity; lethal dose 50; wistar rat, new heterocyclic 1H-perimidine; 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol.

1. INTRODUCTION

The synthesis of new chemicals is worthwhile to be tested for the environmental or health safety. In health condition, toxicology studies could help to make decision whether that new drug should be used for human clinical trial or not [1]. Thus, animal's models are used to a better understanding the toxicological outcomes before determining the pharmacological or therapeutic tolerated doses. The 1H-perimidine system is well known as a major source of novel carbene ligand [2]. This family of product presented a great interest for factories which has been used as a dye and coloring material for polymer [3] and polyester fibers [4]. Regarding its biological activities, it was reported that the components based on perimidines exhibit divers potential beneficial effects on health such as anti-fungal, anti-microbial, anti-tumor among others [4,5]. A past work focused on the synthesis a florescent heterocycle of 1H-perimidine compound 4-(2,3-dihydro-1H-perimidin-2-yl)-2-methoxyphenol) with out testing either lethal dose or specifically biological activities [6]. However, a previous one pointed out that 2-(thiophen-2-yl)-2,3-dihydro-1H-perimidine displayed promising inhibitory activity against acetylcholinesterase as compared to reference drugs including Tacrine [7].

To our knowledge, a new heterocyclic molecule 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxy

phenol belonging to 1H-perimidine chemical group has been synthesized for the first time in good yield at the chemical thermodynamic and physico-chemistry lab of environment (LTPCM) of Nangui-Abrogoua University of Abidjan, Ivory Coast [8]. However, as every new molecules synthesized with some likely therapeutic effect need to be tested for toxicity at the first step, before assaying for its therapeutic power. To this purpose, we study here the acute toxicity with a single intraperitoneal injection of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol, using Wistar rat as experimental model. That could help to appreciate the Lethal Dose 50 (LD 50), the Lethal Dose 100 (LD 100), the Minimal Deadly Dose (MDD) and the NOAEL for this new chemical compound.

2. MATERIALS AND METHODS

2.1 Chemical

The 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxy phenol molecule (Fig. 1) was synthesized and characterized in the thermodynamic and physico-chemistry lab of environment (LTPCM) of Nangui-Abrogoua University of Abidjan (Ivory Coast), using a combination products of Orthovanillin and 1,8-Diaminonaphtalene. The procedure of synthesis and characterization was fully described elsewhere [8].

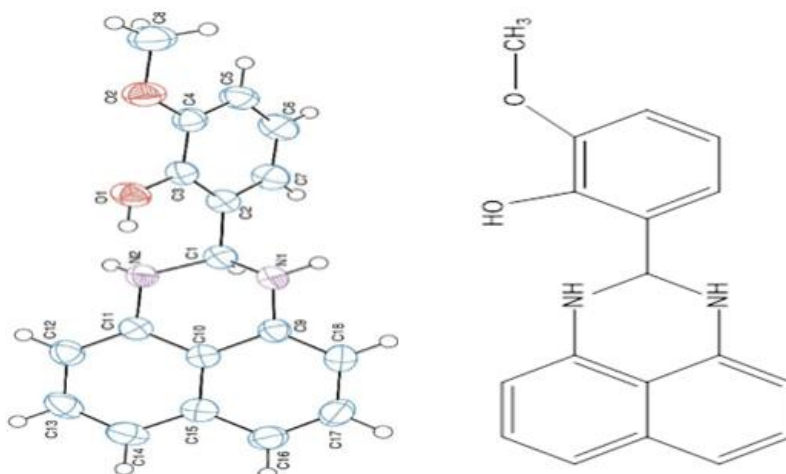


Fig. 1. Chemical structure of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol

2.2 Animals

Current study used male Wistar rats (3-months old) obtained from the animal experimental breeding house of Felix Houphouet Boigny University, Abidjan. The acute toxicity test was carried out according the guideline of Organization for Economic Cooperation and Development (*i.e* OECD). Animals were clinically examined and body weight was individually recorded. The rats weighing around $\pm 20\%$ of the weight mean of all animals was included for acute toxicity regarding OECD recommendation and Canadian Council on Animal Care (CCAC) [9,10]. The relative living environment of animals was kept in estimated standard conditions of temperature (22-25°C) and humidity (60%), and animals had free access to food and tap water. The animals were marked in groups and acclimated for 5 days in home cage before any experiment protocol.

2.3 Experimental Design for Acute Toxicity

The experiment consists of randomly six batches of 5 rats each and a single dose was intraperitoneally injected with 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol compound dissolved in DMSO to every group. The concentrations were ranged from 7 mg/kg body weight (b.w) to 150 mg/kg b.w. enabling to determine the no observable adverse effect level (NOAEL), Minimal Deadly Dose (MDD) which killed the minimum of rats in treated group, lethal dose 50 (LD₅₀) which killed 50% of treated and the lethal dose 100 (LD 100) which killed all of

the rats, after 14 days. The substance was administrated at steady volume of 1 mL / 100 g b.w. The different groups of study was organized as the following:

Batch 1: vehicle rats receive only DMSO solvent by intraperitoneal pathway (1 mL/100 g).

From batch 2 to 6: It was submitted at doses of 7 mg/kg (Dose 1), 40 mg/kg (Dose 2), 90 mg/kg (Dose 3), 120 mg/kg (Dose 4) and 150 mg/kw (Dose 5) of the tested substance in 1 ml of DMSO.

Each animal was observed several times during the first 30 min and more attention was for the following 4 hours of the first day of treatment. Then, animals were inspected once by day for 14 days according the OECD principles. The clinical and acute toxicology signs including changes in skin, eye, mucous membrane, Cardiac and respiratory rhythms, temperature body, hypersalivation, diarrhea, nasal secretion or sleep were observed. Furthermore, some atypical behaviors as convulsion, tremor, posture disturbance and mortality were noted [11]. The food consumption and water intake were daily recorded. Prior all treatment, the animals were weighed and one time by week.

2.4 Statistical Analysis

Statistical analysis was performed the non-parametric test Kruskal-Wallis and multiple comparison of Mean rank (Min-Max) between studied groups. $P < .05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Clinical Signs and Behavior Analysis

From 30 min to 4 hours after the substance injection, we found no case of death in different groups of doses-treated rats. The body temperature was significantly raised to about 38-39°C in rats of batch 6 (temperature data not shown). Intraperitoneal injection of 150 mg/kg b.w induced clinical signs of acute toxicity as tremor at rest, locomotor activity reduction and nasal mucus and that has been continued in rats of batches 3, 4 and 5. The deaths are recorded in rats of lots 3, 4, 5 and 6 correspondent to doses 40, 90, 120 and 150 mg/kg b.w, respectively. Interestingly, 24 hours after the single injection, the dose of 150 mg/kg b.w caused 100% of lethality (LD₁₀₀) (Tab.1). The MDD was 40 mg/kg

b.w. The LD₅₀ between MDD (40% of mortality dose) and 90 mg/kg (60% of mortality dose) was estimated to 65 mg/kg b.w based on the percentage of mortality relative to logarithm of doses curve (Fig. 2). However, all the rats of batch 2 (7 mg/kg b.w) were survived suggesting that corresponds to the NOAEL dose.

3.2 Food Consumption and Water Intake

As depicted on the Fig. 3A, no significant difference of food consumption over the first week post-injection with 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol was found between the different experimental groups. However, the amount of food ingested in rats treated with Dose 2 (40 mg/kg) and Dose 3 (90 mg/kg) was significantly reduced during the second week ($P = .0148$) compared to others.

Table 1. Mortality rate consecutive to 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol doses injection

Batch	Vehicle DMSO (n=5)	Dose 1 (7 mg/kg) (n=5)	Dose 2 (40 mg/kg) (n=5)	Dose 3 (90 mg/kg) (n=5)	Dose 4 (120 mg/kg) (n=5)	Dose 5 (150 mg/kg) (n=5)
Time of death after injection						
30 min	-	-	-	-	-	-
4h	-	-	-	-	-	1
24h	-	-	-	-	2	4
2 days	-	-	-	-	1	-
4 days	-	-	1	1	2	-
7 days	-	-	1	1	-	-
12 days	-	-	-	1	-	-
Mortality (%)	0	0	40	60	100	100

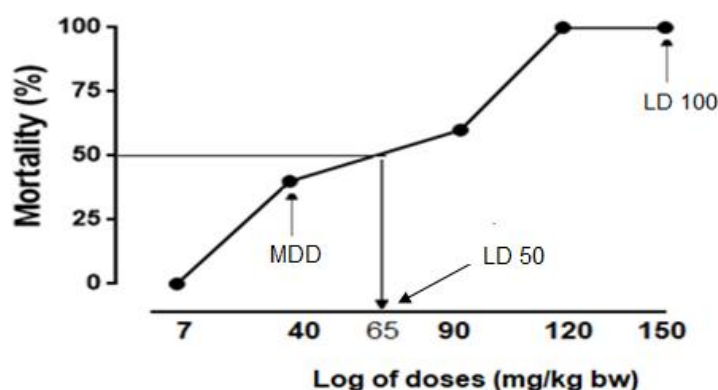


Fig. 2. Curve of mortality rate evolution with logarithm of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol injected

MDD, Minimal Deadly Dose; LD₅₀, Lethal Dose 50; LD₁₀₀, Lethal Dose 100

The level of water intake was decreased in both Dose 2 and Dose 3 groups during the first week ($P < .001$), when compared to others groups. The same observation was done during the second week, unless the level of water drunk by rats of group Dose 3 remained unchanged ($P < .05$), when compared to rats of dose 2 (40 mg/kg) (Fig.3 B).

3.3 Body Weight

The injection of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol at the Dose1 (7mg/kg) did not affected significantly the body weight during the 2 weeks of observation compared to vehicle rats. However, groups of Dose 2 (40 mg/kg) and

Dose 3 (90 mg/kg) showed high significant body weight loss during the first week ($P < .01$), and the second week ($P < .001$) (Fig. 4).

3.4 Liver Appearance

The following images highlight the degree of hepatotoxicity induced by 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol. The LD₁₀₀ dose (150 mg/kg) caused several tumors just few hours after intraperitoneal injection. The knots touched different lobe of the liver, with also some swollen appearance. Regarding the remaining doses, we noticed some necrotic aspects which the spread is reduced with the dose (Fig. 5).

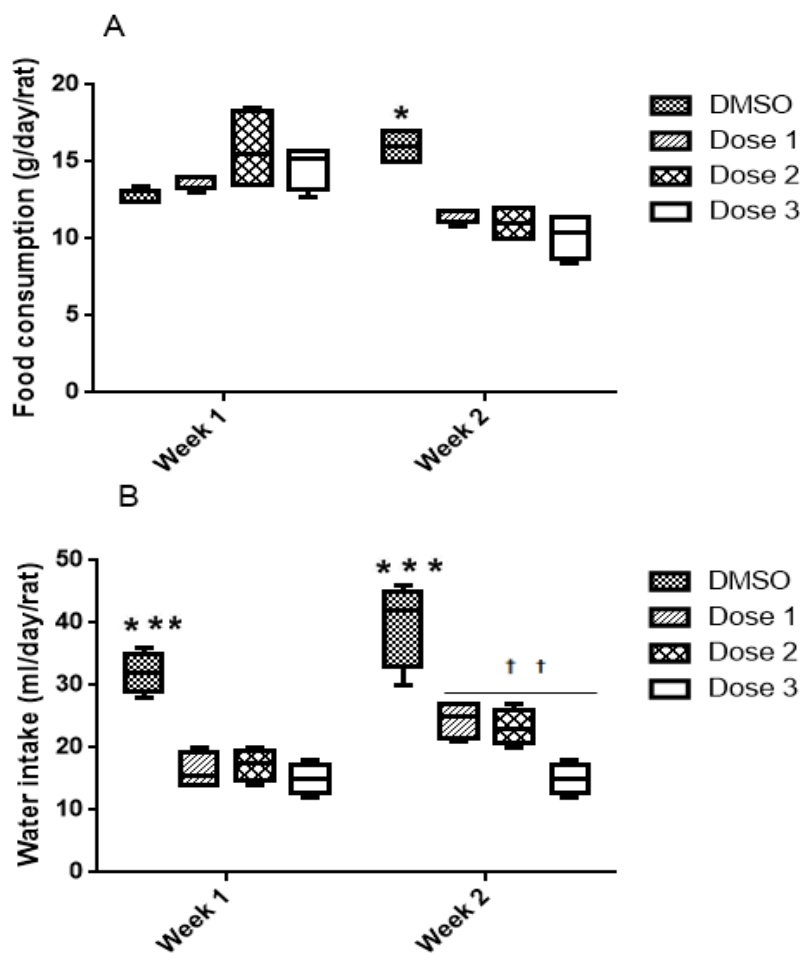


Fig. 3. Food consumption amount (A) and Water intake (B) over two weeks of acute toxicity of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol

Results are presented as mean rang (Min-Max).

A, * $P = 0.05$ (DMSO vs Dose 1, Dose 2 or Dose 3); B, *** $P < .001$ (DMSO or Dose 1 vs, Dose 2 and Dose 3); † $P = .05$ (Dose 1 or Dose 2 vs Dose 3)

DMSO, Vehicle; Dose 1, 7 mg/kg; Dose 2, 40 mg/kg; Dose 3, 90 mg/kg

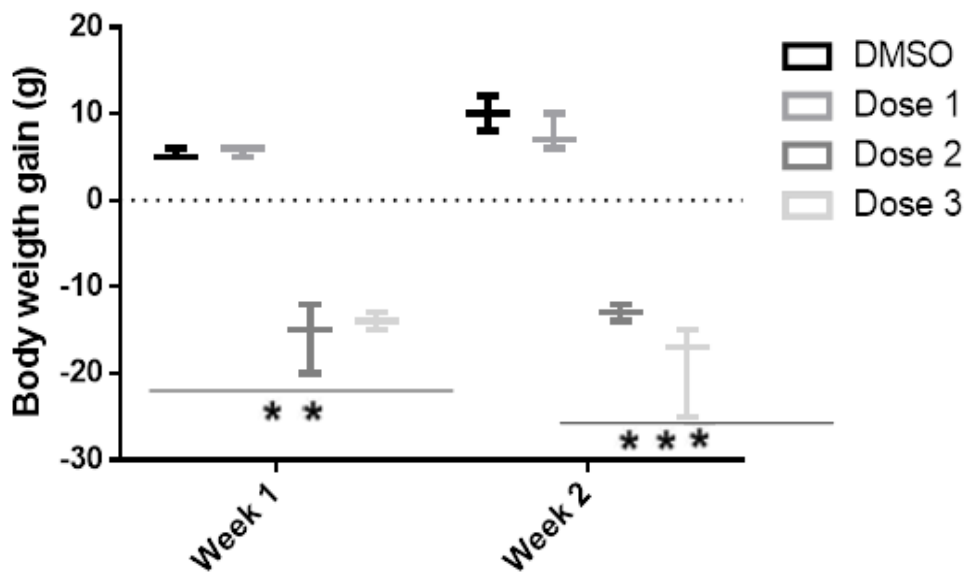


Fig. 4. Body weight gain during experimental weeks

Results are presented as mean \pm SEM (one-way ANOVA/post hoc Tukey)

P < .01 (DMSO or Dose 1 vs Dose 2 and Dose 3); * P < .001 (DMSO or Dose 2 vs Dose 2 and Dose 3)

DMSO, Vehicle; Dose 1, 7 mg/kg; Dose 2, 40 mg/kg; Dose 3, 90 mg/kg

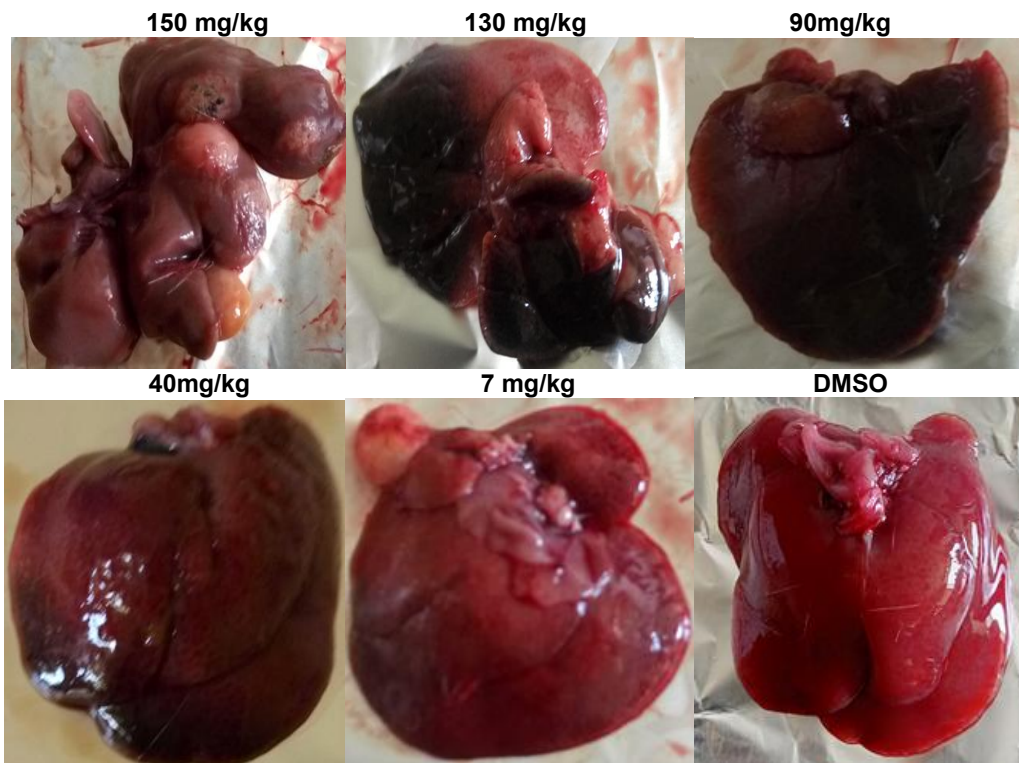


Fig. 5. Liver appearances after injection of rats with 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol

A range of diverse synthesized perimidines and their biological activities as DNA-binding, cytotoxicity properties and anti-tumor activities have been reported [5]. Those authors revealed the fused tricycle perimidine was the minimal structure condition for the intercalative binding with DNA and affected by the variation in side chain of chromophore. Braña et al. demonstrated that the 2-substituted perimidines-induced cytotoxicity action through the presence of basic nitrogen bearing to methyl groups [12]. As new chemicals synthesized, it is worthwhile to be submitted to toxicity studies in order to optimize later the therapeutic doses for heal human diseases. Hence, the acute toxicity study on animal models seem to be the initial step. The acute toxicity of a new product is based conventionally on the indicator of LD50, which is determined according the procedure recommended by OECD (OECD, 2001). Even if, we have previously tested some antimicrobial activities of the new 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol, the acute toxicity has not yet performed. In present study, we aimed to evaluate *in vivo* the deemed LD50 of this new heterocyclic 1H-perimidine and also its effects on behavior. During the 14 days of acute toxicity study, each animal received single dose of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol and monitored day by day until the 14th day, in which we recorded any clinical signs of toxicity [13,14]. Otherwise, as there are no informations about that new product, we selected different doses largely below to 300 mg/kg b.w, the one recommended when giving by oral route [9]. In our study, the intraperitoneal injection of the doses induced dissimilar effects according the injected doses. The highest dose of 150 mg/kg i.p chosen caused 100 % of mortality within 24 hours (LD 100). The dissection of organs revealed several inflammation, necrosis, tumor in the lung, spleen and mainly in liver. It suggests that the dose of 150 mg/kg presents high degree of toxicity which impaired vital functions leading to death. Then we proceeded by lower doses including 130, 90, 40 and 7 mg/kg of bw. We found that the dose of 130 mg/kg i.p killed all animals over four (4) days. Some clinical signs as tremors, salivation, anxiety behavior, underfeeding, and deceleration of cardiac and breathing rhythms have been steadily observed during this period. However, we reported in experimental batches of 90 and 40 mg/kg doses, 60% and 40% mortality rate respectively. To both doses 90 and 40 mg/kg, the mortality was found from 4th to 12th days (Tab.1). Using the logarithmic scale of dose-mortality, the LD 50

has been estimated to 65 mg/kg in Wistar rat. The LD50 reflects the hazardous feature or lethal nature of a new molecule tested, and the new 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol one is considered as product of high toxicity according international toxicity ranking. The duration of action of our chemical was slow in rats of batch 40 mg/kg (Dose 2) which killed a minimal 2 of 5 animals in our experimental condition. The survival rats of the MDD 40 mg/kg recovered fastly from clinical signs when compared to 90 mg/kg (Dose 3). However, we found significant dose-response effects on body weight gain, food consumption and water intake behavior depleted dominantly in the second week in both the rats of Dose 2 and Dose 3 groups. Even if the rats of Dose 3 seem to be more affected. Those results suggest that an injection with 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol could damage endocrinal system and/or disturb the kidney-regulated urine excretion, and that need to be ascertained by an assay of creatinine or urea as biomarkers of renal dysfunction [15]. Otherwise, we noticed that the signs of toxicity lasted few days before killing or recovering. For instance, with dose of 120 mg/kg, 100 percent of animal died from 24th hours to 4th day. However, some rats of doses 40 and 90 mg/kg recovered gradually beyond the 4th day. From these observations, depending on gradual dose received, this new chemical could be accumulated in the body and complicated the pharmacokinetic mechanisms of absorption, metabolism and excretion. That suggests being associated with hepatotoxicity degree highlighted after dissection. In others words, the death could be due to the difficult to eliminate entire level of the product in body.

Interestingly, 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol tested at 7mg/kg is considered as the NOAEL dose in Wistar rat because it did not caused any clinical signs and organs alteration, nor affected feed behavior during the observation period. So, this dose will be useful in non-clinical assessment of the new heterocyclic 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol. In fact, the NOAEL is obviously taking account the basic calculation of the first dose for human safety in clinical study setting, with following formula: NOAEL dose / Safety Factor, Safety Factor ≥ 10 [16].

Otherwise, a previous research showed a series of 1H-2,3-dihydroperimidine derivatives compounds act as new classes of inhibitor of protein tyrosine phosphatase 1B drugs, the

inhibitor percent for two them are around 100% at 20µg/mL [17]. Based on human safety dose of 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol which should not exceeded 0,7 mg/kg and average of blood volume at 60 mL/g b.w, the circulating dose of our new heterocyclic perimidine would be at 11,7 µg/mL. Therefore, the blood concentration of our new heterocyclic could require to be testing *in vitro* for several pharmacology and cytotoxicity properties.

4. CONCLUSION

The present study was addressed to determine different toxic doses dominantly the LD50 and the NOAEL of a new heterocyclic 2-(2,3-dihydro-1H-perimidin-2-yl)-6-methoxyphenol synthesized and characterized by TPCM lab of Nangui-Abrogoua university. As main result, our product presents a high toxicity grade with a LD 50 estimated to 65 mg/kg by intraperitoneal pathway. However, before testing some beneficial effect on health through a chronic study, it is important to analyze the effects on biochemical and hematological parameters, and neurobehavioral ability.

CONSENT

It is not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experimental protocol was carried out according to NIH guide for the care and use of laboratory animals and approved by our local ethic committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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