ACUTE TOXICITY AND *IN VIVO* ANTIOXIDANT ACTIVITY EVALUATION OF AQUEOUS EXTRACTS *FROM PHYLLANTHUS ODONTADENIUS* Müll. Arg.

Abstract

Aims: *Phyllanthus odontadenius* is one of medicinal plants from Phyllanthus genus traditionally used for treatment of a good number diseases including malaria. As indeed for any other drug, *P. odontadenius* could present risks of intoxication for users. This study aims to evaluate the antioxidant activity and acute toxicity in vivo of *P. odontadenius* using rats and mices.

Study Design: *P. odontadenius* plants harvested and dried, plant powders preparation by lyophilization and aqueous extracts preparation, weighed animals (mice's and rats) and drug administration or not for control by gavages'; Monitoring in cages after 7 or 8 days. Animal Sacrifice and Blood draw ; dosage of biochemical parameters (urea, creatinine, AST, ALT, GGT and PAL).

Place and Duration of Study: Division of Life Sciences, General Atomic Energy Commission, Regional Nuclear Studies Center of Kinshasa, P.O. Box. 868 Kinshasa XI (DRC). MPI and pharmacognosy laboratories in the National Biochemical Research Institute (INRB). The experiments were conducted from from October 19, 2021 to January 21, 2022.

Methodology: The aqueous extracts from the aerial parts of *P. odontadenius* harvested in three different sites (Cécomaf/Kinshasa, Kasangulu/Kongo Central and Pont-Kwango/Kwango) were administered respectively to the test mice (2,500 mg/kg of body weight in one dose) and to the test rats (250 and 500 mg/kg body weight each day) after poisoning with lead acetate (50 mg/kg body weight. and distilled water administered to control mice and rats (10 ml/kg body weight). The effects of these extracts on body and organ weights and on biochemical parameters (urea, creatinine, AST, ALT, GGT and PAL) were analyzed.

Results: Regarding acute toxicity, the administration of a single dose of 2500 mg/Kg to mice had no significant effects on body weight as well as on biochemical parameters in mice. The weights of organs like the heart and kidneys were affected in comparison with the mice to the control. Regarding the antioxidant activity *in vivo*, the toxicity effects induced by lead acetate were not visible thanks to the treatment with the aqueous extracts of P. odontadenius, which would justify the presence of the phenolic compounds at the base of the reduction of effects could be caused by the reactive oxygen species (ROS) lead induced.

Conclusion: At the end of this work, it appears that the single dose administration of the 2500 mg/kg does not cause significant signs of toxicity in mice. Treatment of rats with *P. odontadenius* aqueous extracts (250 and 500 mg/kg) after their poisoning reduced the toxic effects due to free radicals caused by lead acetate.

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1. INTRODUCTION

Traditional medicine remains the main recourse of a large majority of populations to solve their health problems, not only because it constitutes an important element of the cultural heritage, but also for the limited financial means compared to conventional products. According to the World Health Organization, nearly 80% of populations depend on traditional medicine for primary health care (WHO, 2009). Almost all cultures and civilizations, from ancient times to the present day, depend wholly or partially on herbal medicine due to their efficacy, accessibility, availability, low toxicity, and acceptability.

For thousands of years, mankind has used various plants found in their environment to treat and cure all kinds of illnesses. Plants represent an immense reservoir of potential compounds attributed to secondary metabolites which have the advantage of being of a great diversity of chemical

structure and possess a very wide range of biological activities. A large number of aromatic plants, spice plants and others have very interesting biological properties which find applications in various fields, namely in medicine, pharmacy, cosmetology and agriculture. These plants represent a new source of active compounds such as phenolic compounds which would play an important role in human health because of their various pharmacological activities such as anti-inflammatory, antiallergic, antimicrobial, antiviral, anticancer, cardiovascular and vasodilatory.

Oxidative stress is involved in a wide spectrum of diseases such as cardiovascular diseases, cancers, diabetes, neurodegenerative diseases (Alzheimer's, Parkinson's) and the aging process. These various diseases are at the origin of enormous complications on the health of populations. Reactive oxygen species (ROS) cytotoxicity is generally controlled by antioxidant systems, substances capable of neutralizing free radicals and thus preventing the onset of diseases associated with oxidative stress. To reduce oxidative stress due to certain pathological effects, the body uses natural antioxidants, enzymatic (SOD, CAT and GPX) or non-enzymatic (carotenoids, flavonoids, vitamins, minerals, etc.), which have the role of extinguish the free radicals generated in the body under various disease states. By default, the restoration of the unbalanced prooxidant/oxidant ratio could be done by synthetic molecules. However, the use of synthetic antioxidant molecules is currently questioned because of the potential toxicological risks. Now, new plant sources of natural antioxidants are being sought (Al-Laith, 2010; Andersson *et al.*, 1996).

P. odontadenius has been shown to have a strong antioxidant potential in the various studies carried out on the in vitro antioxidant activity of its various extracts used, thus corroborating its traditional uses (Kikakedimau *et al.*, 2019; Nsendo *et al.*, 2020). The antioxidant potential of *P. odontadenius* would be explained in its composition in secondary metabolites previously analyzed by various authors and which was found to be rich in phenolic

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compounds (Kikakedimau *et al*., 2021; 2018 and 2013). Phytochemical and pharmacological studies carried out attest to success of *P. odontadenius* in therapy where the extracts of the twigs and leaves reveal antispasmodic, anticancer, antiplasmodial, antidysenteric, antifungal, analgesic properties, etc.

Lead's unique properties (softness, high malleability, ductility, low melting point, and corrosion resistance) have resulted in its widespread use in different industries like automotive, paint, ceramics, plastics, etc. This results in a multiple increase in the presence of free lead in the systems and the inert medium. Lead is considered a powerful occupational toxin and the toxicological manifestations of lead are well known. Lead toxicity is a particularly insidious hazard with the potential to cause irreversible health effects; and it is a much explored and widely published subject. Lead exposure produces various deleterious effects on the different systems such as hematopoietic, renal, and reproductive and central nervous systems, primarily through increased oxidative stress (Flora *et al*., 2012).

Lead-stimulated oxidative stress is a condition that involves the generation of free radicals beyond allowable limits, thereby simultaneously depleting endogenous antioxidant stores and thereby impeding the ability of the biological system to reverse the resulting effects. The generation of free radicals triggers a chain reaction that results in lipid peroxidation, cell membrane disruption, protein oxidation, and oxidation of nucleic acids like DNA and RNA that could lead to cancer (Gurer & Ercal , 2000).

Antioxidants like α-lipoic acid, vitamin E, quercetin and some plant extracts show prophylaxis against lead-induced majority lesions *in vitro* and *in vivo* studies. Generally, an antioxidant can prevent lead toxicity in three ways (Garcia & Gonzalez, 2008): by inactivating the ROS generated at the molecular level, thus terminating the radical chain reaction; by chelating lead ions and preventing the subsequent formation of reactive oxygen species (ROS) such as free radicals superoxide O_2^-, hydroxyl HO^-, Alkoyl RO^- and

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pyroxyl $RO_2°$ and by chelating lead and by keeping it in a redox state, which leads to its inability to reduce oxygen molecules.

Although it has a good antioxidant activity profile *in vitro*, to our knowledge *P. odontadenius* has never been the subject of *in vivo* antioxidant trials in the Democratic Republic of Congo (DRC) and its toxicity remains unknown. It is in this perspective that our study was carried out in order to determine the acute toxicity of aqueous extracts of *P. odontadenius* firstly and, secondly, to determine the antioxidant activity *in vivo* using a proven toxicant which is acetate lead.

Many medicinal plants, such as *Phyllanthus odontadenius* are the rich potential sources in different chemical groups that can protect against damage caused by free radicals. It is with this in mind that samples of *P. odontadenius* were collected in three provinces in order to compare their antioxidant activities.

The main objective of this study is to evaluate the antioxidant activity and acute toxicity *in vivo* of *P. odontadenius*.

This objective should only be achieved when the specific objectives should be achieved beforehand. Among the specific objectives we will retain: the

- collection of samples in the various chosen sites;
- preparation of the aqueous extracts to be administered;
- administration of aqueous extracts in single dose in mice and follow-up;
- Poisoning of rats with lead acetate in a single dose;
- Treatment of rats poisoned with aqueous extracts of *P. odontadenius*;
- Evaluate antioxidant activity and toxicity by assaying a few biochemical parameters including urea, creatinine, transaminases (AST, ALT, GGT) and alkaline phosphatase.

The interest of this study is the *in vivo* orientation, on the one hand, on the beneficial effects of the possible secondary metabolites (antioxidant compounds) that *P. odonadenius* contains in order to prevent lesions caused by free radicals in the body

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especially in case of lead poisoning; and on the other hand, the study of acute toxicity constituting the first stage of safety investigations (IRIEN'GUESSAN *et al*., 2019), thus determining by biochemical analyzes the acute toxicity of *P. odontadenius* plants, finally to the warn their users to guarantee the safe use of this plant.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Study environment and period

The experiment was carried out respectively in two places: first in the life sciences laboratory at the General Commission for Nuclear Energy/Regional Center for Nuclear Studies in Kinshasa (CGEA/CREN-K) at the University of Kinshasa and then, at the MPI and pharmacognosy laboratory at the National Institute for Biomedical Research (INRB) in the Commune of Gombe in Kinshasa town. Plant samples identification and the plant powders preparation to be used in the extracts preparation were done at CGEA/CREN-K. The aerial parts of *P. odontadenius* were used for the various plant powders preparation, their aqueous extracts were used in turn for the evaluation of the antioxidant activity *in vivo* on the Wistar Albino rats strain and for the analyzes of toxicity in NMRI albino mice.

The *P. odontadenius* extracts preparation from different sources (CECOMAF-N'djili/Kinshasa, Kasangulu/Kongo central and Pont – Kwango/Kwango), the rats poisoning and their monitoring in cages, the evaluation of the *in vivo* plant extracts antioxidant activity and biochemical analyzes were carried out at the INRB in the MPI and pharmacognosy laboratory. This study took place during the period from October 19, 2021 to January 21, 2022.

2.1.2. Plant material

The aerial parts of *P. odontadonius* plants harvested in the N'djili river valley in the CECOMAF district (N'djili) in Kinshasa town and those from Kasangulu in the central Congo province and from Pont-Kwango in Kwango province served as plant material for this study. The aerial parts removed were dried in the CGEA/CREN-K life sciences laboratory away from light, humidity and dust. After

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about ten days, these plants were ground using Thomas Scientifique USA brand grinder and the 500 µm powder was obtained which was then stored in well-closed and labeled plastic bottles.

2.1.3. Laboratory animals for experimentation

On the one hand, 20 albino NMRI mice weighing an average of 18.74 grams were used for *in vivo* testing of the toxicological activity of *P. odontadenius* extracts. On the other hand, 32 rats of the Wistar Albinos strain aged 8-9 weeks, in good health and weighing an average of 150 g served as laboratory animals to determine the antioxidant activity of *P. odontadenius* extracts on Wistar rats. The rats were poisoned with lead acetate in order to reduce oxidative stress induced by lead. These animals from the INRB animal facility were subjected to an adaptation period, to the animal facility conditions, to an ambient temperature and natural photoperiod. The animals were reared in plastic cages lined with bedding made of wood shavings. The cages were cleaned and the litter was changed every day until the experiment end. The animals were fed an energetically balanced concentrate.

2.2. Methodology

2.2.1. Preparation of extracts

The aqueous extracts were prepared by decoction. Using a KERN precision analytical balance, 100 grams of powder from the three samples of *P. odontadenius* (Kinshasa, Kasangulu and Pont-Kwango) were weighed and mixed in 1 liter of distilled water. Heating followed until boiling. The decoctions thus obtained are filtered using brand filter paper Wattman number 1 and then collected in a clean bottle. After filtration, the aqueous extract obtained was frozen at -20°C before being freeze-dried using a Christ Alpha LSC 2-4 (Germany) brand freeze-dryer to obtain the dry extract. The latter was used to prepare different dilutions for carrying out antioxidant activity on wistar rats and *in vivo* toxicological tests on NMRI albino mice.

2.2.2. Preparation of solutions (extracts) for use and administration

The antioxidant activity of *P. odontadenius* extracts on Wistar albino rats poisoned with lead acetate at 50

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mg/kg body weight was evaluated using two doses: 250 mg/kg and 500 mg/kg. Vitamin C at 150 mg/kg doses was used as the reference antioxidant (Flora *et al*., 2012). Lead acetate, used as the proven toxicant to induce oxidative stress in rats, was administered to all Wistar rats except those in the control cage which received only distilled water (10 ml/kg body weight) (Etame-Loe *et al*., 2018; Mikolo *et al*., 2020).

The rats were divided into 8 batches of four per cage, including 6 for the two doses (250 and 500 mg/kg) and the last two, one for vitamin C (150 mg/kg) and the other for the control (distilled water, 10 ml/kg).

For monitoring during handling, Wistar rats from all batches were marked with picric acid. The rats of batches 1 – 6 were treated, by gavages' using gastric tube every day for 8 days, either with 250 mg/kg extracts or with 500 mg/kg extracts of each sample. Rats in batch 7 had vitamin C (150 mg/kg) by gavages' using also gastric tube every day for 8 days and those of batch 8 also had distilled water (10 ml/kg) by gavages', using also gastric tube every day for 8 days.

For the acute toxicity study of *P. odontadenius* extracts on NMRI albino mice, the OECD guideline, n°423, (2001), a sequential process using three animals (mice) of the same sex , was used. This method ranks substances in order of toxicity in a similar way. To do this, four (4) batches of five (5) mice each, of the same sex, were formed. Each test mouse received orally, by gavages' using an esophageal probe, single dose of *P. odontadenius* aqueous extract (2500 mg/kg) except for the 5 control mice group which did not have, in one dose, than distilled water (10 ml/kg of weight) as indicated above. The mice were also marked with picric acid in order to follow them individually throughout the experimental period. They were observed at ½, 1, 2, 3 and 4 hours after administration of each product (extracts and distilled water). Mortality was assessed within 48 hours of administration (Mikolo *et al*., 2020).

Observations on the mobility, the color of hairs, as well as on their viability were made during the time of monitoring mice and rats in cages.

2.2.3. Determination of weights

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Previously, the NMRI albino mice and the Wistar albino rats were weighed on the first day of the experiment using KERN brand precision scale in order to monitor their growth in cages according to the products administered to them. To highlight the drug effect on the animal in cages, the percentages of weight gain (increase in mass) are determined by the following relationship:

$$\textbf{\% weight increase} = \frac{P2 - P1}{P1} \times 100$$

Where P1 is the weight of the animal before being subjected to any drug (or treatment) and P2, the weight after treatment of the animal with *P. odontadenius* aqueous extracts for a given time.

2.2.4. Sacrifice and Blood draw

At the end of the treatment, i.e. on the 7th day for the mice and on the 8th day for the rats, the animals are sacrificed in the morning by administering to them ketamine 20 mg/kg of weight to make them fall asleep. All animals in all batches were decapitated using sterile scalpel blades. After each decapitation, blood is taken from the heart in dry Eppendorff tubes. The blood collected is then centrifuged at 2500 revolutions/min for 10 min; the various sera collected were used to determine the biochemical parameters.

2.2.5. Dissection and organ harvesting in mice

After the mice were sacrificed, they were dissected in order to remove some toxic-sensitive organs such as the liver, heart and kidneys. These organs weighed using the KERN brand sion balance. The relative weights of sensitive organs compared to the weights of mice were determined by the following formula (Mikolo *et al.*, 2020):

$$\textbf{Relative weight} = \frac{\textbf{Organ weight (g)}}{\textbf{Animal weight (g)}}$$

Dosage of biochemical parameters

All biochemical parameters (urea, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/GPT), alkaline phosphatase (ALP) and ɣ-Glutamyltransferase (GGT)) were measured thanks to the Cobas C111

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automaton. This device automatically analyzes and calculates the concentration of the biochemical parameter concerned for each sample. The tests are carried out in four stages; these are system start-up, session opening, and system preparation and request creation.

2.2.6. Statistical analyzes

The results are expressed as mean ± standard errors by the Excel software and the variance was studied by Student's "t" test. The significance threshold was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Results

The results obtained, on the one hand, on the *in vivo* toxicological tests of *P. odontadenius* aqueous extracts on NMRI albino mice and on the other hand, on the *in vivo* antioxidant activity of *P. odontadenius* aqueous extracts on rats Wistar are listed in the various tables below.

3.1.1. Growth of caged NMRI Mice

The results on the variation in weight of Albino NMRI mice caged after the single intake of extracts of *P. odontadenius* (2500 mg/kg of weight) according to origin are given in Table 1.

Table 1: *P. odontadenius* aqueous extracts effects on the weights of mice after taking 2500 mg/kg of body weight) and the variations in percentages weight.

Samples extracted P.o	Weight (g) front mouse	Weight (g) mouse after	% Weight increase
Distilled water	19.78±3.08a	22.39±3.87a	13.09
KASANGULU	20.48±1.87a	23.17±2.38a	13.09
KINSHASA	15.92±1.06a	18.00±1.34a	13.07
Pt.-KWANGO	18.8±2.69a	21.26±3.40a	13.09
F-Tab. (5%)	3.11	3.11	-
F-calc.	2.85	2.82	-

It appears from Table 1 that the weights after treatment of the NMRI albino mice varied for all the mice placed in cages, that is to say that there

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was growth of the mice after taking the extracts. The high value (23.17±2.38 g) is found in mice treated with *P. odontadenius* aqueous extracts from Kasangulu and the low value (18.0±1.34 g). However, these values show no significant difference at the conventional threshold of 5%.

3.1.2. Effects of aqueous extracts of *P. odontadenius* on sensitive organ weights

The results on the sensitive organs weights (heart, liver and kidneys) of NMRI albino mice drugged with extracts of *P. odontadenius* (2500 mg/kg of weight) and monitored in cages are shown in Table 2.

Table 2: *P. odontadenius* aqueous extracts effects on sensitive organ weights (g) in NMRI albino mice after dissection.

Samples extracted *P.o*	Weight (g) heart	Weight (g) liver	Weight (g) Right Kidney	Weight (g) Left Kidney
Distilled water	0.12±0.00a	1.01±0.00a	0.19±0.00a	0.17±0.00ab
KASANGULU	0.11±0.01b	1.11±0.09a	0.17±0.02b	0.18±0.03a
KINSHASA	0.09±0.01d	1.00±0.13a	0.11±0.01d	0.11±0.01d
Pt.-KWANGO	0.10±0.01c	1.06±0.09a	0.14±0.02c	0.15±0.02bc
F-Tab. (5 %)	3.11	3.11	3.11	3.11
F-calc.	4.5001125	1.19	10.58	29.086
LSD	0.00016	-	0.028	0.197

Table 2 shows that the high values of the sensitive organs (heart, liver and right and left kidneys) are respectively: 0.12±0.0 g for the control; 1.11±0.09 g for the Kasangulu extract, 0.18±0.03 g for the Kinshasa extract and 0.19±0.0 g for Pont-Kwango). The low values of the sensitive organs (heart, liver and right and left kidneys) are respectively 0.09±0.01 g; 1.0±0.13g, 0.11±0.01g and 0.11±0.01g. All these low values were found in mice treated with the extracts of *P. odontadenius* from Kinshasa. However, there is no significant difference at the conventional threshold of 5% for the liver.

The heart presented significant difference at the conventional threshold of 5% because the value of F-calc. is greater than F-Tab. The weight of the

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heart of the control differs at all the weights of the treatises and these also differ among themselves. The same is true for the kidneys, which also showed a significant difference at the conventional threshold of 5%. The kidney weights of the control are higher than those of the treated.

3.1.3. Relative weights of sensitive organs (heart, liver and kidneys)

The different relative weights of the sensitive organs are listed in Table 3.

It emerges from Table 3 that the relative weights of the sensitive organs generally experienced a decrease compared with those of the control, with the exception of those of the liver, which experienced an increase. However, there is no significant difference at the 5% confidence level between the relative weights of the control and those of hearts, livers and rights of reins. Only the relative weights of the left reins showed a significant difference at the conventional threshold of 5%. The relative weight of the left reins of the control differs from those of the left reins of the rats treated with the extracts of Kinshasa and of Pt-Kwango and not with the relative weight of the left reins of the rats treated with the aqueous extract of Kasangulu.

Table 3. Relative weights of sensitive organs (heart, liver and kidneys) in NMRI albino mice.

Samples extracted P.o	heart relative weight	liver relative weight	right kidney relative weight	left kidney relative weight
Eau dist.	**0.55±0.09a**	**4.61±0.76a**	**0.78±0.13a**	**0.87±0.14a**
KASANGULU	0.47±0.04a	4.82±0.38a	0.78±0.11a	0.74±0.10ab
KINSHASA	0.52±0.03a	5.58±1.10a	0.64±0.10a	0.63±0.10b
Pt-KWANGO	0.49±0.08a	5.08±0.70a	0.69±0.13a	0.66±0.15b
F-Tab. (5 %)	3.11	3.11	3.11	3.11
F-calc.	2.37	1.21	1.91	4.87
LSD	-	-	-	0.149

3.1.4. Effects of *P. odontadenius* aqueous extracts on biochemical parameters in NMRI mice

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The analyzes of the biochemical parameters carried out on the NMRI albino Mice having been subjected to the single administration of the aqueous extracts of *P. odontadenius* (2500 mg/g) are given in Table 4.

Table 4 : *P. odontadenius* aqueous extract (2500 mg/g) effects on biochemical parameters in NMRI Mice after their dissection.

Samples extracted P.o	Urea (mmol/l)	Creatinine (mmol/l)	Urea/ Creat.	SGOT (UI/l)	SGPT (UI/l)	GGT (UI/l)	PAL (UI/l)
Control	12.08±1.40bc	14.8±2.63a	0.82	240.38±62.14a	45.88±3.49a	13.48±3.76a	80.94±12.81a
Kasangulu	16.58±1.51a	14.78±1.79a	1.12	227.66±53.48a	45.00±7.39a	3.87±0.29b	71.78±11.23a
Kinshasa	14.53±2.14ab	14.62±1.86a	0.99	226.94±41.34a	49.40±6.48a	3.68±1.23b	71.78±11.38a
Pt -Kwango	16.31±2.07a	14.74±1.49a	1.12	153.68±75.36a	34.74±4.79b	6.9±3.87b	76.54±10.60a
F-Tab (5%)	3.11	3.11	-	3.11	3.11	3.11	3.11
F-calc.	5.56	0.0104	-	2.148	6.12	6.12	0.6112
LSD	2.712	-		-	7.86	4.1073	-

It appears from Table 4 that of the renal balance parameters, urea presented values which increase for the mice having received extracts of *P. odontadenius* (2500 mg/kg) compared to that of the control (12.08±1 .40 mmol/l) unlike creatinine which presented values close to each other.

Mice treated with Kasangulu *P. odontadenius* aqueous extracts showed high mean urea value with 16.58±1.51 mmol/l compared to the control. Statistically, the urea values show a significant difference at the 5% confidence level between the control value and the values of urea dosed in mice having been intoxicated with extracts of *P. odontadenius* (2500 mg/g) from Kasangulu and those of Pt.-Kwango but not with the value of urea assayed in mice having received extracts of *P. odontadenius* from Kinshasa.

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For creatinine, the high value is that of the control with 14.8±2.63 mmol/l and the low value (14.62±1.86 mmol.l) is that of the mice treated with the Kinshasa extracts. However, the creatinine values show no significant difference at the conventional threshold of 5%. With regard to the ratios of the average values of urea and those of creatinine, the low value is that of the control compared to the other ratios which surround the unit.

With regard to Table 4, it appears that the SGOT values decreased in the mice which had the *P. odontadenius* aqueous extracts (2500 mg/kg) compared to the mean SGOT value of the control (10 ml/kg of distilled water) with (240.38±62.14 IU/L). The low SGOT value is that of NMRI mice treated with the Pt.-Kwango extract (153.68±75.36 IU/L). However, there is no significant difference at the 5% confidence level between the SGOT values of the caged mice.

Table 4 shows that the values of the parameters of the liver balance sheet of the NMRI mice intoxicated with *P. odontadenius* aqueous extracts (2500 mg/kg) and monitored in cages decreased for ALAT, GGT and PAL compared to the control. The values of SGPT and GGT showed a statistically significant difference at the 5% confidence level. On the other hand, the PAL values of did not show any significant difference between them at the 5% confidence level.

3.1.5. Growth of Wistar rats in cages

The results of the growth of the caged rats expressed by the variation of the weights before the toxic intake (lead acetate 50 mg/kg of body weight (bw) and after their treatment with *P. odontadenius* aqueous extracts (250 and 500 mg /kg bw) are listed in table 5.

It can be seen from Table 5 that post-treatment weights increased for all caged rats throughout the treatment time. After treatment, the high value (237.15 ± 44.19 g) is found in rats treated with Pt-Kwango *P. odonatednius* aqueous extracts and the low value (174.2 ± 65.73 g) in the control (distilled water, 10 mg/kg). However, these values do not show a significant difference at the conventional threshold of 5% because the value of F-calc.

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(0.54) is lower than that of F-tabular (3.11).

Table 5: Effect of *P. odontadenius* aqueous extracts (250 and 500 mg/g bw) on the weights of Wistar albino rats poisoned with lead acetate (50 mg/kg bw).

Samples extracted P.o	Front weight	weight after	Weight difference (g)	% weight increase
KIN_250	176.98±16.79a	197.98±8.26a	21.00±9.64a	11.87
Pt-KGO_250	169.28±20.74a	199.06±12.57 a	21.10±5.59a	12.47
KASA_250	212.00±73.11a	217.94±77.63 a	16.07±7.91a	7.58
Vit. C	178.15±7.24a	203.48±12.78 a	30.13±9.50a	**16.91**
Eau dist.	144.08±63.15a	174.20±65.73 a	25.33±7.46a	**17.58**
KIN_500	202.60±30.32a	227.78±32.50 a	25.18±4.69a	12.43
Pt-KGO_500	208.15±25.14a	237.15±44.19 a	29.00±24.87a	13.93
KASA_500	176.98±16.79a	197.98±8.26a	21.43±14.41a	12.11
F-Tab. (5%)	3.11	3.11	3.11	-
F-calc.	2.38	1.33	0.54	-

The growth percentages (weight increase) show a high value for the control (17.58%) followed by that of Vit. C (16.91%) compared to the percentages of weights obtained in rats treated with *P. odontadenius* aqueous extracts (250 and 500 mg/kg). The weight difference also did not show a significant difference at the conventional threshold of 5%.

3.1.6. Effects of aqueous extracts of *P. odontadenius* on biochemical parameters in albino Wistar rats

3.1.6.1. Kidney parameters: Urea and creatinine

The results on the analyzes of urea and creatinine and their ratios after treatment of rats with *P. odontadenius* aqueous extracts (250 and 500 mg/kg) to reduce oxidative stress due to lead acetate (50 mg/ kg bw) are listed in table 6.

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Table 6 shows that urea values increased in all Wistar albino rats poisoned with lead acetate (50 mg/kg bw) and treated with *P. odontadenius* aqueous extracts (250 and 500 mg/g bw) compared to the control (distilled water at 10 mg/kg bw) and those who had vitamin C (150 mg/kg bw). The high urea values (10.13±0.65 mmol/l and 11.15±2.05 mmol/l) are found in Wistar albino rats treated with Pt.-KWANGO *P. o* extracts and the low value is that of the control (distilled water at 10 mg/kg) with 8.19±1.98 mmol/l. However, all these values show no significant difference at the conventional threshold of 5%.

For creatinine, the mean values generally also increased except in Wistar albino rats treated with *P. o* extracts from Pt.-KWANGO and Kinshasa at a dose of 500 mg/kg bw. The high value is that of vitamin C with 34.68±1.96 mmol/l. However, there is no significant difference at the conventional threshold of 5%.

3.1.6.2. Biochemical parameters (ASAT/SGOT, ALAT/SGPT, GGT and PAL)

Table 6: Effect of *P. odontadenius* aqueous extracts (250 and 500 mg/g bw) on urea and creatinine in Wistar albino rats poisoned with lead acetate (50 mg/kg bw).

Samples extracted *P.o*	Urea (250) mmol/l	Creatinine (250) mmol/l	U250/C250	Urea(500) mmol/l	Creatinine (500) mmol/l	U500/C500
Kinshasa	9.41±0.54a	34.7±2.51a	0.27	9.21±1.41a	31.2±1.28a	0.31
Pt-Kwango	10.13±0.66a	32.58±2.04a	0.31	11.15±2.05a	28.48±2.96a	0.39
Kasangulu	9.35±0.91a	32.87±3.03a	0.28	8.94±0.74a	34.13±1.92a	0.26
Vit. C	9.35±0.55a	34.68±1.96a	0.27	9.35±0.55a	34.68±1.96a	0.27
Eau dist.	**8.19±1.98a**	**31.9±3.13a**	**0.26**	**8.19±1.98a**	**31.9±3.13a**	**0.26**
F-Tab. (5%)	3.11	3.11	-	3.11	3.11	-
F-calc.	1.29	0.0011	-	1.03	0.46	-

The results on the dosage of SGOT, SGPT, GGT and PAL after treatment of rats with *P. odontadenius* aqueous extracts (250 and 500 mg/kg)

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to reduce oxidative stress due to lead acetate (50 mg/kg of pc) are listed in Table 7.

Table 7: Effects of *P. odontadenius* aqueous extracts (250 and 500 mg/g bw) on SGOT, SGPT, GGT and PAL in Wistar albino rats poisoned with lead acetate (50 mg/kg bw).

Samples extracted P.o	SGOT (UI/l)	SGPT (UI/l)	GGT (UI/l)	PAL (UI/l)
KINSHASA (250)	182.50±50.51a	70.65±6.53a	1.58±0.10a	**185.73±66.08a**
Pt-KWANGO (250)	**127.20±21.51a**	70.00±4.97a	2.50±0.14a	196.80±64.13a
KASANGULU (250)	173.40±20.15a	**62.97±7.47a**	2.13±0.25a	244.97±81.80a
Vit. C (150 mg/kg)	**174.48±95.43a**	**52.28±11.12a**	**1.15±0.57a**	**214.43±92.55a**
Eau distillée	**133.68±9.57a**	**65.40±13.51a**	**1.38±0.49a**	**230.95±86.13a**
KINSHASA (500)	147.45±34.73a	66.25±13.62a	2.38±1.50a	255.03±94.95a
Pt-KWANGO (500)	137.20±18.20a	67.63±10.53a	1.73±0.66a	251.83±93.88a
KASANGULU (500)	133.83±9.58a	63.13±6.98a	1.50±0.37a	289.58±71.40a
F-Tab. (5%)	3.11	3.11	3 .11	3.11
F-calc. (250 mg/kg)	0.11	0.013	0.85	0.79
F-calc. (500 mg/kg)	1.087	0.497	0.2068	1.061

With regard to Table 7, it appears that the values of the biochemical parameters of the cardiac assessments are high compared to the values of the control except for the average values of SGOT resulting from the analyzes carried out on the albino Wistar rats treated with Pt – Kwango *P. odontadenius* extracts (250 mg /kg) and PAL from analyzes carried out on Wistar albino rats treated with *P. odontadenius* extracts (250 mg/kg) from Kinshasa and Pt – Kwango. The high values are respectively 174.48±95.43 IU/L (Vit. C) for SGOT, 70.65±6.53 IU/L (Kinshasa 250) for SGPT, 2.50±0.14 IU/L (Pt – Kwango 250) for GGT and 289.58±71.40 IU/L (Kasangulu 500) for PAL. However, all the values of the biochemical parameters analyzed do not show any significant difference from each other at the conventional threshold of 5%.

DISCUSSION

Quantitative analysis of certain enzymes in blood plasma has diagnostic

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importance in different pathological processes. Other enzymes are released into plasma following cell death or injury. Although having no physiological function in plasma, the presence of these enzymes or concentration can guide the diagnosis and prognosis of diseases and wounds affecting certain tissues.

Among the main serum enzymes used for clinical diagnosis are transaminases or aminotransferases including aspartate aminotransferase (ASAT or SGOT) for the diagnosis of myocardial infarction, alanine aminotransferase (ALAT or SGPT) for viral hepatitis, γ-glutamyl-transferase (GGT) for various liver diseases and alkaline phosphatase (ALP) for the diagnosis of various bone conditions and obstructive liver diseases. However, it should be noted that several of these enzymes are not specific for the disease mentioned above (Murray *et al.*, 2013).

Species of the genus Phyllanthus have traditionally proven themselves in the treatment of a number of diseases including malaria, dysentery, fungal diseases and many others (Asare *et al.*, 2011; Yeap *et al.*, 2021). Studies carried out in the laboratory to prove the relevance of the use of Phyllanthus species extracts including *P. odontadenius*, from gamma irradiation (Cs-137) or not, soaked in sodium azide (NaN_3) or not, on the antiplasmodial activities *in vitro* have proved to be promising (Kikakedimau *et al.*, 2014; Kikakedimau *et al.*, 2017 and Luyindula *et al.*, 2018). The various studies on phytochemical screening and on the antioxidant activity of aqueous or methanolic *P. odontadenius* extracts *in vitro* have sufficiently demonstrated that this plant contains many compounds such as phenolic compounds what are found in significant quantities. These are the basis of the justification of a good and significant in vitro antioxidant activity of Phyllanthus genus plants including *P. odontadenius* (Kikakedimau *et al.*, 2019; Nsendo *et al.*, 2020).

The toxic effects of a substance are the result of biochemical interactions between the substance and/or its metabolites and the structures of the organism to which it is administered, probably justifying the almost constant evaluation of biochemical and hematological parameters during

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toxicological studies (IRIEN'GUESSAN *et al.*, 2019). This is where this study is justified in order to understand the acute toxicity and *in vivo* antioxidant activity of *P. odontadenius*, which until now seems to have never been studied.

The clinical use of a substance of therapeutic interest is always preceded by a toxicity test (acute, subacute, sub-chronic and chronic) in order to establish the incurred risk during its administration (Fané, 2002 reported by IRIEN 'GUESSAN *et al.*, 2019). The variations in blood concentrations of urea and creatinine remained within the range of normal values for all batches, i.e. 0.15 to 0.45 g/l for urea and, i.e. 65 - 120 µmol/l. However, there was an increase in urea concentration in NMRI albino mice after the intake of 2500 mg/kg body weight compared to the control (distilled water 10 mg/kg body weight). Serum urea and creatinine are considered the main markers of nephrotoxicity, although serum urea is often considered a more reliable predictor of kidney function than serum creatinine (Etame *et al.*, 2018). Together with the increase in serum urea, this would explain some nephrotoxicity due to the high dose of aqueous extract of *P. odontadenius*.

In Wistar albino rats, variations in urea concentration (8.19±1.98 to 10.13±0.66 for 250 mg/kg bw and from 8.19±1.98 to 11.15 ±2.05 mmol/l for 500 mg/kg bw) and creatinine (31.9±3.13 to 34.7±2.51mmol/l for 250 mg/kg bw and 31.9±3.13 to 34.68±1.96 mmol/l for 500 mg/kg bw) did not show any significant difference although there are increases which would be due to lead acetate and which would be more still high had it not been for the treatment with *P. odontadenius* aqueous extracts. This could be a justification for the role played by *P. odontadenius* extracts as an antioxidant in traditional treatment. We can argue that the increase in urea levels in rodents treated with lead acetate could be explained by an increase in the catabolism of protein compounds (Hajjaj, 2017).

The calculation of the urea/creatinine ratio is useful in acute renal failure because it is found to be high in pre-renal insufficiency and in acute urinary obstruction, which helps to distinguish these pathologies from acute renal failure of the type renal and

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chronic urinary obstruction, conditions where the ratio does not change since the urea is not reabsorbed in an increased way (Clinilab - November 2000). The urea to creatinine ratio is normally between 10:1 and 20:1. The values of the urea to creatinine ratios obtained in this study varied from 0.82 to 1.12 in NMRI albino mice and from 0.26 to 0.39 in Wistar albino rats. The decrease in the urea to creatinine ratio can be observed during heart disease or dehydration. It can also be observed during the increase in protein, in the event of gastrointestinal bleeding or during a diet rich in protein. This ratio can also be reduced during liver pathologies (by reduction in urea formation) or in the event of malnutrition (Anonymous, 2022).

Hepatic dysfunction is followed by elevated levels of serum enzymes (AST, ALT and PAL), indicating cellular leakage and loss of functional integrity of the hepatic membrane. The liver constitutes an important target of lead; the latter has a great affinity for the protein thiol group of the hepatic cell membranes, which leads to hepatic necrosis and following the release of transaminases into the serum. These results are confirmed by the work of Gurer and Ercal (2000) (SOUAD, 2016).

The results obtained in this study show increased activities in AST (133.68±9.57 to 182.50±50.51 IU/L), ALT (52.28±11.12 to 70.65±6.53 IU/L) and PAL (214.43±92.55 to 289.58±71.4 IU/L) in the serum of rats treated with lead acetate, thus justifying the effect of this toxique in relation to the witness. However, these are values that did not have a significant difference and this would also justify the antioxidant activity of *P. odontadenius* aqueous extracts. On the other hand, in NMRI albino mice, there was only the significant difference with AST; the other two parameters, ALT and PAL, did not show a significant difference. The percentage of weight gain shows that lead acetate acts effectively on poisoned rats treated with *P. odontadenius* extracts (250 and 500 mg/l) because all treated rats percentages (7.58% to 13 91%) are lower than that of the control (17.58%) and vitamin C (16.91%).

The results obtained on the various parameters (urea, creatinine, GGT, ALAT, ASAT and PAL) by ASARE

et al. (2011) who worked on the acute toxicity of *Phyllanthus niruri* aqueous extract on female Sprague-Dawley (S-D) rats showed that there was no significant difference either for the dose of aqueous extracts at 2000 mg/kg body weight than at 5000 mg/kg body weight. Our results on the same biochemical parameters (Tables 4, 6 and 7) corroborate those of Asare *et al*. (2011) in Wistar albino rats poisoned by lead acetate and treated with P. odontadenius extracts at doses of 250 and 500 mg/kg body weight. On the other hand, in NMRI albino mice subjected to a single dose of 2000 mg/kg of body weight, significant differences were found for three biochemical parameters including urea, ALT and GGT. These different changes could be explained by the difference in secondary metabolites that each Phyllanthus species would contain and in the physiological behavior of the used experiment animals.

Yeap *et al*. (2021) reports that *P. tenellus* extracts aqueous (1000 mg/kg body weight) significantly reduced serum levels of PAL, ALT and creatinine compared to control mice. On the other hand, a preliminary study of short-term acute toxicity carried out reported that *P. tenellus* ethanol extract up to 2500 mg/kg of body weight did not induce mortality in mice and that no significant change in serum creatinine level in mice treated with *P. tenellus* extract (3000 mg/kg body weight) was observed compared to untreated normal control mice. Our results generally support the results obtained by Yeap *et al*. (2021) in the reduction of the biochemical parameters analyzed (SGOT, SGPT, GGT and PAL) and that also the serum level of creatinine in mice treated with the aqueous extract of *P. odontadenius* (2500 mg/kg of body weight) did not showed a significant change at the conventional 5% threshold. During the treatment time of 7 days, there were no dead mice.

Murray *et al*. (2013) reports that GGT also used for the diagnosis of various liver diseases, this transaminase was increased in the serum of treated rats poisoning with lead acetate. In this study, the GGT values varied from 1.38±0.49 to 2.50±0.14 IU/L and these values increased slightly. However, no significant difference was observed. The

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action of the antioxidant activity of aqueous extracts of *P. odontadenius* would be the basis of the reduction of oxidative stress due to lead acetate. In NMRI albino mice, on the other hand, the GGT with values ranging from 6.9±3.87 to 13.48±3.76 IU/L showed a significant difference compared to the control and these values decreased compared to the control. A low or normal level of GGT indicates that liver damage is very unlikely or it shows that the liver and to a lesser degree the muscles have not been affected. (Koné and Bleyere, 2009; BRAUN, 2021).

ALTs are more specific for liver damage, but ASTs are somewhat more sensitive (Goddard and warnes, 1992). Nevertheless, for some authors such as Charrel reported by Fall *et al.* (2011), during an imprecise clinical picture, it is possible to admit with virtual certainty a hepatic component when the serum ALT activity is greater than that of the ASATs. Contrary, our results both in NMRI albino mice having received *P. odontadenius* extracts (2500 mg/kg of body weight) or in Wistar albino rats poisoned with lead acetate (50 mg/kg of body weight), AST values were found to be higher than ALT values. Transaminases or amino-transferases are tissue enzymes that catalyze the transport of alpha-amino radicals from alanine and aspartic acid to alpha-ketoglutaric acid. Present in liver, but also in muscle and in kidney, pancreas, and other tissues, ASTs are synthesized in the cytoplasm of the cells of these organs and released into the circulation when these cells are damaged (Peirs, 2005 cited by ETAME *et al.*, 2018).

Upadhyay *et al.* (2009) demonstrated that rats fed food contaminated with lead undergo a state of oxidative stress linked to a serum elevation of transaminases (ASAT, ALAT) and alkaline phosphatases (ALP). Our results support this thought in the sense that AST, ALT and PAL values generally increased in Wistar albino rats poisoned with lead and treated with *P. odontadenius* extracts compared to the control. However, there was no significant difference between control and treated values. This phenomenon is due to the accumulation of amino acids such as alanine and glutamic acid resulting from the degradation of somatic proteins. These

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amino acids are transformed under the action of transaminases into ketone bodies and then into glucose (gluconeogenesis).

CONCLUSION

The purpose of this study was to determine, on the one hand, the acute toxicity of *P. odontadenius* extracts *in vivo* and, on the other hand, to justify the use of *P. odontadenius* in a traditional way by determining the antioxidant activity in vivo by use of a proven toxique.

It turned out that *P. odontadenius* would present a possible toxicity in NMRI mice at the dose of 2500 mg/kg of body weight by the fact that the weights of the organs like the heart and the kidneys are affected in the direction of the decrease. The biochemical parameters showed variations in SGPT (ALAT) and GGT in the direction of reduction, but these are changes that would not have effects on the liver or on the heart. Body weights did not change significantly.

The positive effects of *P. odontadenius* aqueous extracts were shown on the antioxidant activity *in vivo* to oxidative stress induced by lead acetate because all the parameters analyzed (body weight, urea and creatinine, transaminases; ASAT, ALAT , GGT and PAL) showed no significant changes. But weight growth was initiated in rats poisoned with lead acetate compared to non-poisoned rats (weight growth: 17.58%) because the percentages of weight growth decreased significantly for rats poisoned and treated with *P. odontadenius* aqueous extracts.

CONSENT

The Laboratories analysis obtained the consent for use laboratories animals.

ETHICAL APPROVAL

The ethical approval is obtained by the National Institute for Research Biomedicine for works on laboratories animals.

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