GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Acute oral toxicity evaluation of hydroethanolic extract from stem and leaf powder of *A. annua* (Asteraceae) in laboratory rats

Rosine Désirée Nkuitchou-Chougouo [1, 2, *], Landry Kouakou [3], Ernest Djoko [1], Guy Rocard Tchiesso [1], Théophile Dimo [4] and Armand Angely Koffi [2]

[1] *Pharmacy Department, Faculty of Health Sciences, Université des Montagnes, Bangangté Cameroon.*
[2] *Department of Galenic Pharmacy and Biopharmacy, Faculty of Pharmacy and Biological Sciences, Felix Houphouet Boigny University, Abidjan, Ivory Coast.*
[3] *Department of Pharmacology, Faculty of Pharmacy and Biological Sciences, Felix Houphouët Boigny University, Abidjan, Ivory Coast.*
[4] *Department of Animal Biology, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon.*

GSC Biological and Pharmaceutical Sciences, 2022, 21(02), 230–241

Publication history: Received on 13 October 2022; revised on 22 November 2022; accepted on 25 November 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.21.2.0441

Abstract

Introduction: Development of a new galenic form containing vegetables extracts for paediatric use could be a good therapeutic alternative to overcome chemoresistance and therapeutic failures observed with conventional antimalarial drugs. The aim of this work was to verify in laboratory rats, the toxicity of hydroethanolic extract from stem and leaf powder of *A. annua* (Asteraceae), in order to provide scientific evidence to support its use in rectal dosage forms preparation.

Methodology: Acute toxicity assessment was possible by performing OECD Test Guideline 425, 2008 limit test in laboratory rats. Examination of spontaneous exploratory activities and motor coordination was respectively realized according to methods described by Colman, 2015 and Deacon, 2013. In addition, we monitored body weight changes, food and water consumption including the impact of extract on relative organs weight such as liver, kidney, lung and heart.

Results: Single administration of *A. annua* stem and leaf extract at 5000mg/kg was not toxic in rats, even less to liver, kidney, lungs and heart functions. Elsewhere it was noted that consumption of *A. annua* extract significant increase curiosity and motor activity (number of lines crossed: 76.50 s ± 45.30; grasping time: 92.25 ± 38.85) compared to control (number of lines crossed: 54.00 ± 20.78 s; grasping time: 51.00 s ± 20.28).

Conclusion: Extract of stems and leaves of *A. annua* at 5000mg/kg can be considered non-hazardous and non-toxic category 5 according to the Harmonised System of Classification of Toxic Substances.

Keywords: Safety*; In-vivo*; *Artemisia annua*; Plant extract; Aerial parts

1. Introduction

Malaria is a fatal human parasitic disease transmitted to humans by bite of infected mosquitoes (*female anopheles*) [1-3]. Drugs commonly used to treat this disease are subject to chemoresistance allowing resurgence of therapeutic failures which poses a real public health problem [4-8]. Face to this problematic find anti-malarial from plants could be an

* Corresponding author: Chougouo-Nkuitchou K Rosine D
Pharmacy Department, Faculty of Health Sciences, Université des Montagnes, Bangangté Cameroon.

alternative to fight this pandemic, especially with the discovery and isolation of artemisinin (*Artemisia annua)*, a medicinal plant from traditional Chinese pharmacopoeia [9-13].

Artemisia annua is a plant that grows well in tropical countries [14-16] like in the city of Bangangté (Cameroon) and various scientific studies have demonstrated its effectiveness against malaria, including chills and fever. (17-19). In addition, this plant has anti-hemorrhoidal [20, 21] and antitumour properties [22].

The laboratory of University of Montagnes (Cameroon)/Department of Galenic Pharmacy and Biopharmacy of UFHB (Ivory Coast), had developed new thermogelific form for paediatric use. Before conducting clinical studies to confirm efficacy of this product against malaria, it was important to verify the safety of plant extracts, so that our study proposed to evaluate in laboratory rats' acute oral toxicity of hydroethanolic extract from powdered stems and leaves of *A. annua* (Asteraceae) in order to provide scientific evidence that would justify its safe use.

2. Material and methods

2.1. Methods

2.1.1. Plant Material

Stems and leaves of *Artemisia annua*, was the areal part of this plant used for the study. Seeds used for cultivation came from MEDIPLANT (Research Centre for Medicinal and Aromatic Plants CH 1964 Conthey/Switzerland) [23]. The plant was harvested in January 2015 in Bangangté town, NDE department, and West Cameroon region. Plant material was identified in national herbarium by comparing with an existing sample under identification number 65647/HNC.

Areal Plant parts were harvested as soon as flower buds appear [24], and then cleaned under running water to remove impurities. With secateurs, leafy stems were separated from other plant parts and dried in shade after spreading at an ambient temperature of 30 ± 2 °C for 14 days, then in an oven at 60 °C for 2 hours, in order to avoid thermodegradation of bioactive compounds (including artemisinin) [24]. Stems and leaves were cut into small pieces of 2 to 3 cm and introduced into a propeller mill to obtain a coarse powder that was passed through 300 µm diameter mesh sieve to select particles for produce extracts under study (Figure 1).

(Photographed by TCHIESSO Guy Rocard 20/12/2015)

Figure 1 Fine powder of stems and leaves of *Artemisia annua*

2.1.2. Experimental animals

Male and female (nulliparous and non-pregnant) albinos' rats (Rattus norvegicus) used for this study weighing between 150g and 180g (figure 2). These animals were acclimatised in animal house of "Université des Montagnes" (Bangangté town), under controlled environmental conditions (residual humidity: 82.15 ± 9.91%; mean temperature: 21.91 ± 0.99°C) with a 12-hour light and 12-hour dark cycle. Daily animals diet consisted of 80% maize, 10% crushed dry fish, 3% groundnuts, 2% eggshells, 1% roasted soybeans, 0.1% multivitamins and 3.9% palm kernel meal.

(Photographed by TCHIESSO Guy Rocard 20/12/2015)

Figure 2 Wistar albino rats

2.1.3. Chemicals

- Distilled water (University of the Mountains Multipurpose Laboratory)
- Ethanol 96°C (POLYPHARMA®, Bafoussam, Cameroon)
- Alcohol 70°C (Laboratory of Galenic Pharmacy of the University of the Mountains)
- Ether (Cooper®)
- NaCl 0, 9% (POLYPHARMA®, Bafoussam, Cameroon

2.2. Methods

2.2.1. Preparation of solutions

Preparation of the hydroethanolic macerate of the powder of the stems and leaves of *Artemisia annua*.

Hydro-ethanolic macerate of *Artemisia annua* from stem and leaf powder was prepared with 50 mg of plant powder mixed with 150 mL of hydro-ethanolic solution (30:70 v/v) for 24 hours at room temperature. After filtration through wattman paper No.3, extract obtained was collected in 60ml hermetically sealed bottles and stored in a refrigerator at 7 °C.

2.3. Toxicity test

Acute oral toxicity of *A. annua* stems was assessed in rats following the limit test of OECD Test Guideline 425 adopted in 2008 [25-27].

2.3.1. Principle

Study consisted of looking signs of toxicity in rats that had previously received by gavage a single dose of hydroethanolic extract from stems and leaves of *A. annua* at 5000 mg/kg. The animals were observed for 24 hours after administration and then every 2 days until 14th day compared to control group.

Animals were examined for coat appearance, gaze fixation, noise reactivity, tremor, convulsions, staggering gait, exophthalmos, stool appearance and death.) In addition, four hours (4h) after gavage, spontaneous exploratory activity, muscle tone and animals motor coordination were assessed, including weight gain (7ième and 14ième days), food and water intake (6th and 13th days) [25]. Extract evaluated would be non-toxic in absence of any sign of toxicity, whereas death or presence of harmfulness signs in animal would indicate toxicity [28].

2.3.2. Experimental protocols

Eight female rats were fasted for 6 hours before experimentation were divided into two weight-homogeneous groups of 4 rats each. Using a blunt-ended oesophageal tube, animals were given 1ml/100g b.w. of solutions as follows:

- Group 1 (Control: NaCl): consisting of rats that received 0.9% saline (0.9% NaCl);
- Group 2 (Test: *A. annua* 5000 mg/kg): consisting of rats that received the hydroethanol extract of the stems and leaves of *A. annua* at 5000 mg/kg

Mortality rate percentage was determined using following formula:

$$\text{Mortality } \% = \frac{Number\ of\ dead\ treated\ rats}{Number\ of\ treated\ rats} \times 100$$

2.4. Spontaneous activity test

Method used was described by Colman, 2015 [29].

2.4.1. Principle

Open enclosure arena illuminated, measuring 80 cm on each side and 60 cm in height, has an exploratory surface consisting of 24 squares divided into squares of 16 cm each [30, 31] (Figure 3).

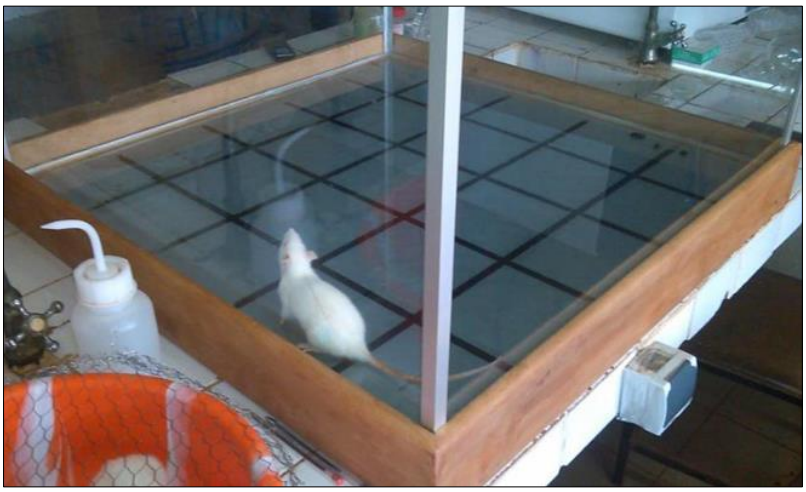

Figure 3 Open arena photographed by (TCHIESSO Guy Rocard on 15/10/2015)

Four hours after administration of *Artemisia annua* extract, animal was placed in center of experimental set-up and observed for 5 minutes. Spontaneous locomotor activities such as number of crossing lines, rearing, grooming, number and appearance of stools produced and time spent in center of arena were recorded. After passage of an animal, experimental device was cleaned with ethyl alcohol (70°) to eliminate odours [29]. When spontaneous locomotor activity was increased, extract improved animal's curiosity, while the extract inhibited this activity.

2.5. Grip test

The method used was described by Deacon R., 2013 with some modifications [30].

2.5.1. Principle

Test consisted of measuring duration in seconds of animal's suspension when it was placed vertically by its forelegs on a cylindrical metal bar (60 cm long, 5 mm in diameter and 40 cm high) placed horizontally [31, 32] (Figure 4).

When duration of the grip is long, extract would be toning, on the other hand extract would inhibit muscle tone.

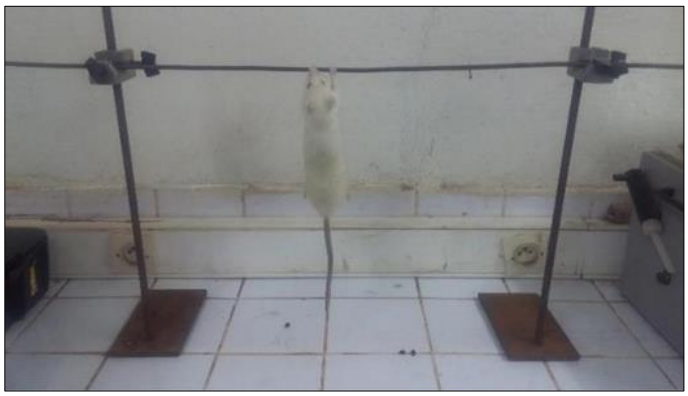

Figure 4 Grip test device (photographed by TCHIESSO on 18/11/2015)

2.6. Bridge crossing test

Method used was described by Deacon R., 2013 with some modifications [30].

2.6.1. Principle

The test consisted of measuring time in seconds taken by animals after administration of *Artemisia annua* extract, to reach one end of a horizontal bar once the animal was placed above and in the middle of metal bar [31, 33] (Figure 5).

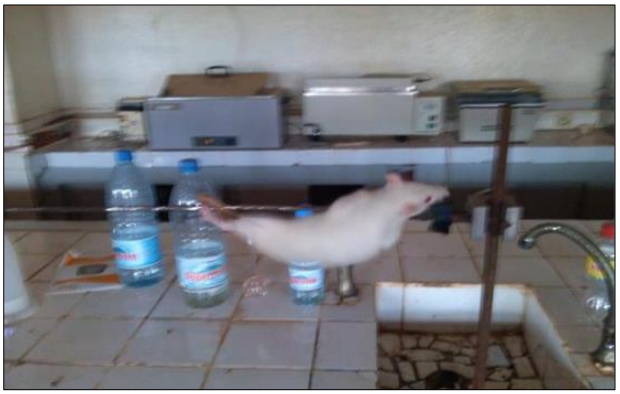

Figure 5 Crossing test (photographed by TCHIESSO on 18/11/2015)

When duration of the crossing is rapid, extract would promote motor skills, while on the other hand the extract would inhibit motor skills.

2.7. Weight evaluation

Monitoring weight evolution was possible by determining weight of each animal in percentage per week according to following formula:

$$Weight\ change\ = \frac{(\mathrm{PMM}\ -\ \mathrm{PMJSP})\ \times\ 100}{\mathrm{PMJSP}}$$

WBW: weight at time of measurement

PMJSP: weight on same day of measurement but from previous week

When weight increases, extract stimulates appetite, while on the other hand extract inhibits appetite.

2.8. Food and water consumption evaluation

2.8.1. Principle

Food and water consumption were assessed as a percentage (i.e., grams per 100 grams of body weight per day and milliliters per 100 grams of body weight per day, respectively) using the following formula:

$$CH\ ou\ CA\ = \frac{\text{CHRJ ou CARJ}\ \times\ 100}{\text{Poids de l'animal}}$$

CHRJ: water consumption in millilitres for one rat per day
CARJ: food consumption in grams for one rat per day
CA: food consumption in grams per 100 grams of rat per day
CH: water consumption in millilitres per 100 grams of rat per day

Each animal was placed in an empty cage where it had free access to 50g of feed and 25mL of water. After 24 hours, amount of feed (after sieving) and water remaining was determined, and the food and water intake per gram of body weight per day was determined.

2.9. Relative weight organs evaluation

Animals were euthanised to facilitate organ removal. Relative weights of the liver, kidney, lung and heart of each animal were calculated as a percentage using the following formula:

$$PR\ = \frac{\text{PO}\ \times\ 100}{PA}$$

PR: relative weight of an organ
PO: weight of the organ
AP: weight of the animal at the time of sacrifice

When relative weight of organ was increased or decreased, extract would have a toxic effect on organ, while in contrast, extract would be non-harmful.

Data analysis and processing

Data were entered in an Excel 2013 and results obtained were represented as means with standard deviation (SD). These data were analysed using Graph Pad Prism software (version 8.0.2). Comparison of means was carried out by non-parametric Kruskal-wallis test, and differences observed were statistically interpreted at risk $\alpha = 5\%$ (if $p > 0.05$: not significant difference; if $p < 0.05$: significant difference; if $p < 0.01$: highly significant difference).

3. Results

3.1. Toxic effects of *Artemisia annua* extract after single administration

Table 1 summarises toxicity signs after a single oral dose of *Artemisia annua* at 5000 mg/kg

No deaths or symptoms of toxicity were observed at 5000 mg/kg during 14 days observation.

3.2. Effects of *Artemisia annua* extract on spontaneous exploratory activity, muscle tone and motor coordination

Table 2 Summarises spontaneous exploratory activity, muscle tone and motor coordination after administration of a single dose of *Artemisia annua* extract at 5000 mg/kg.

After administration of single dose of *Artemisia annua* at 5000 mg/kg, the results revealed that the number of lines crossed (NLT), and the grabbing time (TG) were significantly increased compared to control group.

Table 1 Toxidrome after oral administration of *Artemisia annua* extract at 5000 mg/kg

Observed parameters	Witness	5000 mg/kg	2h		4 h		48h		Day 4		Day 6		Day 8		Day 10		Day 12		Day 14	
			Witness	5000 mg/kg	Witness	5000 mg/kg	Witness	5000 mg/kg	Witness	5000 mg/kg	Witness	5000 mg/kg	Witness	5000 mg/kg	Witness	5000mg/kg	Witness	5000 mg/kg	Witness	5000 mg/kg
Aspect	m		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
coat																				
Salivation			A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Locomotion			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trembling			A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Aspect		of	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
faeces																				
Exophthalmos			A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion			A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Approach			A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
faltering																				
Reactivity		at	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
noise																				

Mean values (n = 4) were expressed on a visual scale with following codes: Absent; G: Gritty; +: Normal mobility; -: Reduced mobility; --: More reduced mobility; Normal appearance and P: Presence

Table 2 Effects of *Artemisia annua* on spontaneous exploratory activity, muscle tone and motor coordination

Lots	NLT		NC		NT	TPC (s)	NSE	TT (s)	TA (s)	
Witness	51.00	± 20.28	0.00	± 0.00	2.00 ± 0.81	4.25±2.27	2.00±1.00	3.75±1.25	54.00	± 20.78
A. annua										
5000mg/kg	76.50	± 45.30**	0.00	± 0.00	2.75 ± 1.40	10.50±6.59	1.50 ± 0.69	3.25 ± 0.50	92.25	± 38.85**

Kruskal-wallis test: Values expressed as mean ± standard **deviation; risk α = 5%, n = 4;** NLT: Number of Lines Traversed; NC: Number of Cambrages; NT: **Number of Groomings; TPC: Time;** Spent in Centre; NSE: Number of Saddles Released; TT: Traversal Test and T**A: Grasping Test.; ****: Significant difference compared to control: NLT *A. annua* 5000mg/kg (p =0.0231) ; TPC *A. annua* 5000mg/kg (p =0.0442); TA *A. annua* 5000mg/kg (p =0.0112)

3.3. Effects of *Artemisia annua* extract on body weight

Table 3 showed weight variation after 14 days of observation.

Table 3 Effects of *Artemisia annua* at 5000 mg/kg on body weight

Lots	Weeks

	Week 1	Week 2
Witness	4.36 ± 1.73	3.9 ± 2.86
A. annua 5000mg/kg	7.4 ± 2.83	3.6 ± 1.95

Kruskal-wallis test: Values expressed as mean ± standard deviation; risk α = 5%, n = 4; Week 1: *A. annua* 5000mg/kg (p = 0.065); Week 2: *A. annua* 5000mg/kg (p =0.059); No significant weight change was observed (p ≥ 0.05) in treated group compared to control.

3.4. Effects of *Artemisia annua* stem and leaf powder extract on food and water intake

Table4 showed quantity of feed and drink consumed by animals after 14 days of observation

Table 4 Effect of *Artemisia annua* extract on food and water quantity consumed

Days	Food consumption in grams per gram of rat per day		Water consumption in millilitres per gram of rat per day	
	Witness	*A. annua* 5000mg/kg	Witness	*A. annua* 5000mg/kg
Day 5	0.09 ± 0.01	0.12 ± 0.04	0.09 ± 0.01	0.05 ± 0.03
Day 12	0.10 ± 0.01	0.13 ± 0.07	0.09 ± 0.01	0.05 ± 0.04

Kruskal-wallis test: Values expressed as mean ± standard deviation; risk α = 5%, n = 4.; Day 5: *A. annua* 5000mg/kg (p = 0.0699); Day 12: *A. annua* 5000mg/kg (p = 0.0721); Values represent mean ± standard deviation, n = 4; No significant quantity changes of food and water consumed were observed in treated rats compared to control (p ≥ 0.05).

3.5. Effects of *Artemisia annua* extract on relative organ weight

Table 5 showed impact of *Artemisia annua* on vital organs of the rat.

Table 5 Effects of *A. annua* on vital organs

Lots	Heart	Lungs	Left kidney	Right kidney	Liver
A. annua					
5000mg/kg	0.32 ±0.03	0.78 ± 0.13	0.33 ± 0.02	0.35± 0.02	2.76± 0.39
Witness	0.38 ±0.07	0.84 ± 0.06	0.34 ± 0.02	0.35 ± 0.03	2.69± 0.29

Kruskal-wallis test: Values expressed as mean ± standard deviation; risk α = 5%, n = 4.; Day 5: *A. annua* 5000mg/kg (p = 0.0583); Day 12: *A. annua* 5000mg/kg (p = 0.0637)

Relative masses of organs were not significantly different compared to control (p ≥ 0.05). Macroscopic observation of treated rats (appearance, presence or absence of nodules, necrosis) revealed no physical changes in liver, kidneys, lungs and heart.

4. Discussion

Artemisia annua is a plant with many effective properties to fight malaria including chills and fever.

This plant originating from Chinese medicine is on increase interest for researchers and the aim of this work was to verify experimentally on rat the safety of the hydroethanolic extract of *A. annua* (Asteraceae) from stem and leaf powder, in order to provide scientific evidence of its safe use in humans.

The single administration of *A. annua* stem and leaf extract at 5000mg/kg was beneficial as no signs of toxicity were detected, and no physical damage affected considerable organs such as liver, kidney, lungs and heart. Elsewhere it was noted that consumption of the extract resulted in a significant increase in curiosity and motor activities.

Our results are similar to Chuipet, 2012 who reported that the infusion of *Artemisia annua* stem and leaf powder [40] was more than 10 g/Kg, twice the dose we evaluated in our study i.e. 5000 mg/kg. Mukinda et al, 2007; reported that oral LD50 of extracts of *Artemisia afra*, a plant of a same family we evaluated but a different species, was around 8960 mg/kg [41]. A similar result on same plant *Artemisia afra* but close to results we obtained was also verified by Ketema et al., 2020; specifying that the LD50 of this plant was higher than 5000 mg/kg on his evaluation concerning acute and subacute toxicity of aqueous extract from leaves of *Artemisia afra* in Swiss albino mice [42].

According to Harmonised System of Classification of Toxic Substances, and view of these results, we can affirm that extract from powdered stems and leaves of *A. annua*, was not dangerous and non-toxic category number 5 (28).

As for weight gain, food and water consumption, our results differ from those obtained by Emmanuel et al, 2014 team who observed these parameters decrease after treatment with an extract of *A. annua* leaves [43]. The explanation would reside in composition of powder used to prepare extracts. Emmanuel et al., 2014 used only leaf extract, whereas in our study extract consumed by animals was a mixture from stems and leaves of *A. annua*.

Preliminary phytochemical screening of *Artemisia annua* stem and leaf powder revealed the presence of tannins, alkaloids, saponosides, terpenes, sterols, flavonoids, coumarins and reducing sugars. Similar studies conducted by other investigators have found identical results compare to ours [44, 45]. However, environmental conditions, harvest time and storage conditions of *Artemisia annua* could influence the presence of these secondary metabolites in the plant. This hypothesis was verified by Ashok et al. 2013, who noted an absence of alkaloids in methanolic extract of *Artemisia annua* leaves [44]. Similarly, work of Chougouo et al., 2016 supported this assertion because this author proved that variability in chemical composition of *Artemisia annua* depending on the ecosystem [46] in which this plant grown.

5. Conclusion

In view of these results and according to Harmonized System of Classification of Toxic Substances, we can affirm that extract from stem and leaf powder of *A. annua* was not dangerous and not toxic and authorise us to categorise his toxicity on number 5.

Compliance with ethical standards

Acknowledgments

All authors are thankful ''Université des Montagnes'' Bangangté Cameroon and faculty of pharmaceutical sciences for their valuable support in carrying out this work.

Disclosure of conflict of interest

Authors declare that they have no conflict of interest.

Statement of ethical approval

Experimental procedures were conducted in strict compliance with care and use guidelines for laboratory animals of European Union statements.

References

[1] World Health Organization. Terminologie OMS du paludisme. mise à jour 2021. Genève. Disponible sur: https://apps.who.int/iris/handle/10665/351089

[2] Djitrinou S. , Bankole H. , Atchade P. , Tchogou P. , Medeton J. Variation de la prévalence du paludisme chez les malades consultant l'hôpital de zone d'Aplahoué de mai à juillet 2019. Disponible sur: http://biblionumeric.epac-uac.org:8080/jspui/handle/123456789/1795

[3] Delcus C. Le paludisme, première maladie vectorielle au monde. Aide-Soignante. 2017;31(186):16-7. 2017;31(186):16-7.

[4] Arjen M. Artemisinin Resistance in Plasmodium falciparum Malaria - NEJM. The New England Journal of Medicine. 30 Jul 2009; 455-67.

[5] Plowe C. Malaria chemoprevention and drug resistance: a review of the literature and policy implications. Malar J. Dec 2022;21(1):104.

[6] Noedl H. , Se Y. , Schaecher K. , Smith B. , Socheat D. , Fukuda M. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med. 11 Dec 2008; 359(24):2619-20.

[7] Ashley E., Dhorda M. , Fairhurst R. , Amaratunga C. , Lim P. , Suon S. , et al. , et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 31 Jul 2014; 371(5):411-23.

[8] Assani A. La lutte contre le paludisme en Côte d'Ivoire: directives internationales et pratiques médicales (1948-1996) [Thèse PhD]. [Paris]: Université Panthéon-Sorbonne - Paris I; Université Félix HouphouëtBoigny (Abidjan, Côte d'Ivoire),; 2017.

[9] Tang W., Eisenbrand G. Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. Springer Science & Business Media; 2013. 1005 p.

[10] Wright C., Linley P., Brun R. , Wittlin S., Hsu E. Ancient Chinese methods are remarkably effective for the preparation of artemisinin-rich extracts of Qing Hao with potent antimalarial activity. Molecules. 2010;15(2):804-12.

[11] Shahrajabian M., Sun W., and Cheng Q. Exploring *Artemisia annua* L., artemisinin and its derivatives, from traditional Chinese wonder medicinal science. Not Bot Horti Agrobot Cluj-Napoca. 22 Dec 2020; 48(4):1719-41.

[12] Vellotis C. Drug Toxicity: New Research. Nova Publishers; 2008. 350 p.

[13] Wagner H., Hikino H., Farnsworth N. Economic and Medicinal Plant Research. Academic Press; 2012. 161 p.

[14] Chougouo-Kengne R., Kouamouo J., and Mouyou-Somo R. Etude comparative de l'efficacité thérapeutique de l'Artesunate seul ou en association avec l'amodiaquine et de la tisane de *Artemisia annua* cultivée à l'Ouest du Cameroun. Ann Pharm Université Kinshasa RDC. 4(1):129-50.

[15] Tu Y. Chapitre 7. Études sur la culture et le développement de *Artemisia annua* L. In: De *Artemisia annua* L aux artémisinines. EDP Sciences; 2021. p. 189-214

[16] Blanc B., Bernard W., and Jean-Pierre N. Réflexions autour de la culture d'*Artemisia annua* et de la production d'artémisinine. Ethnopharmacol Doss Spéc Parasitoses Trop. 2008;(41):82-7.

[17] Akchiche A. Mahamdeoua N., Bouchenak B., Boussouf L. Les plantes médicinales à activité antipaludique [PhD Thesis]. University of Jijel; 2008.

[18] Inter-Cultural. Artemisia and the fight against Covid19 [Internet]. Inter-Cultural. [cited 2 Aug 2022]. Available from: https://inter-culturel.net/artemisia-et-lutte-contre-le-Covid-19.html

[19] Eklu-Natey Raphaël D., Balet Annie (in collaboration with M. A. Ahyi, E. J. Adjanohoun, L. Ake Assi, F. Borst, C. Chatelain, D. Diallo, K. Hostettmann,

[20] Sanou, M. Koumaré). Pharmacopée africaine, Dictionnaire et monographies multilingues du potentiel médicinal des plantes africaines. Afrique de l'Ouest. J Afr. 30 juill 2013;1(83-2):202-5.

[21] Van der Kooy F., Sullivan S. The complexity of medicinal plants: The traditional *Artemisia annua* formulation, current status and future perspectives. J Ethnopharmacol. 28 Oct 2013;150(1):1-13.

[22] Icipe K. Whole-leaf *Artemisia annua*-based antimalarial drug: report on proof-of- concept studies. Nairobi: Int Cent Insect Physiol Ecol. 2005;

[23] Liu N., Qu L., Xiang B. , Yang R. Antitumor mechanism of Qinghaosu derivatives--molecular docking studies of Qinghaosu derivatives with transferrin. Yao Xue Xue Bao. Acta Pharmaceutica Sinica, 27 Nov 2009;44(2):140-4.

[24] Chougouo-Nkuitchou R. N'Guessan A., Cécile N., Ernest D., Jonas K., Pierre T. Formulation de Gélules à Base de Feuilles et de Tiges d'*Artemisia annua*. Health Sciences and Disease, 2020;21:8.

[25] Elfawal M., Towler M., Reich N., Golenbock D., Weathers P., Rich S. Dried Whole Plant *Artemisia annua* as an Antimalarial Therapy. Plos one. 20 Dec 2012;7(12):e52746.

[26] Organisation pour la Coopération et le Développement Economique OCDE. Ligne directrice 425. Paris. 2008..

[27] Christian N., Elysée E., Pierre N., Yinyang J., Manaoda A., Adiogo D. Assessment of the Acute Toxicity of the Venom of the Kind of Tetraponera in the Rats. Saudi J Pathol Microbiol, 2022, vol. 7, no 7, pp. 329-332.

[28] Moussa G., Mama K., Jean-Baptiste G., Elisée K., Ulrich K., et al. Etude de la toxicité aigüe d'un extrait éthanolique des tiges de Massularia acuminata (Rubiaceae) chez le rat wistar. International Journal of Innovation and Applied Studies, 2022;35(2):9.

[29] Government of Canada, Canadian Centre for Occupational Health and Safety. Globally Harmonized System (GHS): OSH Answers [Internet]. 2022 [cited 31 Oct 2022]. Available from:https://www.cchst.ca/oshanswers/chemicals/ghs.html

[30] Colman AM. A Dictionary of Psychology. Oxford University Press; 2009. Available from: https:// www.oxfordreference.com /view/10.1093/acref/9780199534067.001.0001 /acref-9780199534067

[31] Deacon R. Measuring motor coordination in mice. Journal of Visualized Experiments. 29 May 2013;(75):2609.

[32] Jacquez B., Choi H., Bird C., et al. Characterization of motor function in mice developmentally exposed to ethanol using the Catwalk system: Comparison with the triple horizontal bar and rotarod tests. Behavioural brain research, 2021, vol. 396, p. 112885.

[33] Pallier P., Drew C. Morton A. The detection and measurement of locomotor deficits in a transgenic mouse model of Huntington's disease are task- and protocol-dependent: influence of non-motor factors on locomotor function. Brain Res Bull. 30 March 2009;78(6):347-55.

[34] Louhimies S. Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. Alternatives to Laboratory Animals, 2002, vol. 30, no. 2_suppl, pp. 217-219.

[35] Combes R. and Balls, M. Comments on the sub-group reports of the EU technical expert working group on the revision of directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. Alternatives to Laboratory Animals, 2007, vol. 35, no. 1, pp. 155-175.

[36] Hartung T. Comparative analysis of the revised Directive 2010/6106/EU for the protection of laboratory animals with its predecessor 86/609/EEEEC-a t4 report. Altex-Alternatives to animal experimentation, 2010, vol. 27, no 4, pp. 285-303.

[37] Davies H., Nutley S., and Mannion R. Organisational culture and quality of health care. BMJ Qual Saf. 1 June 2000;9(2):111-9.

[38] Bloomsmith M., Perlman J., Hutchinson E., et al. Behavioral management programs to promote laboratory animal welfare. Management of animal care and use programs in research, education, and testing, 2017, pp. 63-82.

[39] Kennedy B. and Froeschl K. Education and Training. Management of Animal Care and Use Programs in Research, Education, and Testing, 2017, pp. 221-265.

[40] Veissier I. Animal experimentation: biological, ethical and legislative elements. Prod Anim Fr [Internet]. 1999 [cité 18 oct 2022];

[41] Chuipet N. Etude préliminaire à l'utilisation d'une phytothérapie à base d'*Artemisia annua* chez les enfants de 6 mois à 5 ans : dosage de l'artémisinine et étude de la toxicité de la tisane et de la limonade. Thèse d'exercice. Université des Montagnes-Cameroun; 2011. Disponible sur: La Vie Re-Belle: https://lavierebelle.org/?page=pdf&id_document

[42] Mukinda J. Syce J. Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents. J Ethnopharmacol. 30 May 2007;112(1):138-44.

[43] Mekonen K, Afework M., Makonnen E., et al. Evaluation of Acute and Sub-Acute Toxicity of Aqueous Extracts of Artemisia afra Leaves on Brain, Heart and Suprarenal Glands in Swiss Albino Mice. Ethiopian Journal of Health Sciences, 2020, vol. 30, no. 6.

[44] Ogbole E., Isaiah I., Ogundeko T., Asalu A., Builders M., Aguiyi J. Acute toxicity studies of locally cultivated *Artemisia annua* leaf extract in Rats. World

[45] J Pharm Sci, 2022; 2 (1864-1870). Available at: http://localhost:8080/xmlui/handle/123456789/657

[46] Praveen K. Kumud U. Preliminary Phytochemical Screening and Physico-ChemicalParameters of Artemisia absinthium and *Artemisia annua*. Joural ofPharmacognosy and phytochemistry. 2013.

[47] Ekiert H. , Świątkowska J., Klin P., Rzepiela A., Szopa A. *Artemisia annua* - Importance in Traditional Medicine and Current State of Knowledge on the Chemistry, Biological Activity and Possible Applications. Planta Med. Jul 2021;87(8):584-99.

[48] Nkuitchou-Chougouo R., Fomekong D., Kouamouo J., Hansen G., Lutgen P., Flies M. Physicochemical Characteristics of *Artemisia annua*, an Antimalarial Plant from the Grass- field Regions of Cameroon. Int J Eng Sci Comput. March 2016;6(3):2762-9.