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Research Article

Safety assessment of *Markhamia tomentosa* (Benth.) K. Schum. (Bignoniaceae) leaves extracts, highlight the psychostimulant effect of the methanol extract

Romeo Joel Guemmogne Temdie[1,2*], Guy Bertrand Kamdem[2], Marc Germain Minoue Kuum[2], Flaure Donfack Metchi[2], Antoine Sanda KadaA[3], Joseph Njiaza[2], Agathe Lambou Fotio[4], Théophile Dimo[2]

[1] *Department of Biological Sciences, Faculty of Science, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon*
[2] *Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon*
[3] *Department of zoology and animal physiology, Faculty of Science, Faculty of Science, University of Bamenda, P.O. Box 39, Bambili, Cameroon*
[4] *Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon.*

*Correspondence should be addressed to Roméo J. Guemmogne Temdie: temdie2011@gmail.com, Tel: + 237 696926462

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Abstract

Markhamia tomentosa is a medicinal plant used for the prevention and treatment of various diseases. Studies have demonstrated analgesic and anti-inflammatory activities of aqueous and methanol extracts of this plant's leaves. However, despite its wide use, complete toxicological data are not available. Therefore, this study was designed to assess the acute innocuousness of the aqueous and methanol leaves extracts of *M. tomentosa*. Experimental animals were given a single dose of aqueous or methanol leaves extracts of *M. tomentosa* (2000 or 5000 mg/kg), following a stepwise OCED protocol. Treated animals were submitted to an observation period of 14 days. Mortality, adverse clinical signs, anthropometric parameters, and necropsy discoveries were carefully recorded. The behavioral and neuromuscular alterations were assessed in the open field, bar holding, and bridge tasks. The result revealed that rats treated with aqueous or methanol leave extract of *M. tomentosa* did not exhibit any treatment-related abnormal proven medical signs of toxicity during 14 days, moreover, no mark of necrosis and no death were recorded within this period. The growth rate, as well as feeding, were normal within the observation period. The methanol extract of *M. tomentosa* induced an increase in the number of crossings in open field task, reduced the suspension latency of rat with their forelimbs in bar holding test, and also diminish the latency to cross the bridge, demonstrating that it may possess a psychostimulant effect. Postmortem examinations did not display any noticeable abnormalities. Results suggest that, at 5000 mg/kg body weight, aqueous or methanol leaves extract did not disclose any treatment-related adverse side effects. However, a slight psychostimulant effect of the methanol extract was recorded at the dose of 2000 mg/kg. These findings demonstrated that these extracts may be relatively non-toxic, with LD_{50} greater than 5000 mg/kg.

Key words: *Markhamia tomentosa,* acute toxicity, LD_{50}, rats, psychostimulant effect.

1. Introduction

Drugs from medicinal plants have been used against several human diseases for thousands of years (Abubakar *et al.,* 2018). This social practice which concerns an increasing number of populations especially in developing countries, is nowadays considered as a substitute treatment besides modern medicine (Fawzi, 2013). The extensive use of herbal medicines in low-income countries is undoubtedly due to its availability and affordability in comparison to modern medicines (WHO, 2019). Compounds originated from plants and their derivatives are the main sources of drugs for, it has been demonstrated that at least 64% of the standardized drugs contain active principles initially isolated from medicinal plants (Haidan *et al.,* 2016). Another argument in favor of the great attention for phytotherapy is that people wrongly believe in the safeness and harmlessness of natural and frequently used drugs from plants, especially when the toxicity of such compounds was not pointed out (Loha *et al.,* 2019). Thus, international regulatory authorities require the safety evaluation of herbal products through different pertinent guidelines, which have been framed to acquire toxicological information, before these medicinal plant products should be granted authorization for commercialization (WHO, 2019). Henceforth, the safety and efficiency of drugs and plant products ought to be studied thoroughly to maximize their benefits for mankind. To attain this purpose, a toxicological evaluation should be performed following the internationally accepted guidelines, published by regulatory authorities, which provided a procedure for selecting safe doses for human uses (Nélida, 2018). Among various prescribed testing protocols, the Organization for Economic Cooperation and Development (OEDC) testing guidelines, are the best compared to other traditional approaches, because, they are progressively actualized to fit scientific exigency (OECD, 2001b).

M. tomentosa is largely distributed in the tropical, sub-tropical regions (Shahina & Ghazanfar, 1989). Some medicinal usage of this plant against chest pain, headache, lumbago, edema, and gout (Arbonnier, 2002), legs edema, and elephantiasis of the scrotum, canker, rheumatic pain, diseases of the respiratory tract, bouts of swamp-fever (Bouquet & Debray, 1974; Irvine, 1961), constipation, fever, general pain, headache or backache (Ainslie, 1937), snakebite/venom, sore eyes, heart pain (Adebajo *et al.,* 2007), ailments of the reproductive system (Bep, 1986) is greatly documented. Many studies demonstrated that different extracts from *M. tomentosa* have a wide range of pharmacological activities, such as antimalarial activities of the ethyl-acetate extract and antiprotozoal activities of some of its components (Tantangmo *et al.,* 2010), larvicidal activities of the methanol extract (Adebajo *et al.,* 2007). Analgesic and anti-inflammatory activities of aqueous and methanol extracts (Temdie *et al.,* 2012) and antiarthritic activities (Temdie *et al.,* 2016) of this plant have been demonstrated as well. Likewise hepatoprotective effects of the methanol extract of *M. tomentosa* on D-galactosamine/lipopolysaccharide-induced acute liver injury have been assessed (Temdie *et al.,* 2020). Phytochemical studies revealed the presence of saponins, tannins, anthraquinones, alkaloids, glycosides, cardiac glycosides, flavonoids and phenols in the methanol leave extract of *M. tomentosa* (Temdie *et al.,* 2012) and 2-acetylnaphtho[2,3-b]furan-4,9-dione and 2-acetyl-6-methoxynaphtho[2,3-b]furan-4,9-dion were isolated from its ethyl-acetate leave extract (Tantangmo *et al.,* 2010).

Despite the wide exploitation of the *M. tomentosa* in traditional medicine and notwithstanding the demonstrated properties of its extracts, only a few data concerning its toxicological profile are available. The

high toxicity of some constituents of the ethyl-acetate extract on the myeloblast of rat's skeletal muscle was established (Tantangmo *et al.,* 2010). Out of this data on cytotoxicity, we have conducted a sighting study on the methanol leaves extract of the *M. tomentosa* in other to seek for harmful effects of this extract. For this purpose, a single dose of 300, 1000, or 5000 mg/kg was administered orally to Swiss albino mice (one mouse per dose level) and these mice were subjected to an observation period of 7 days. Treated mice did not die during the observation period (data not shown). This information indicates that these extracts are likely to be non-toxic. OEDC protocol prescribes in this circumstance that, a limit test should be carried out, at a dose level of 2000 mg/kg, for a safety evaluation of acute oral toxicity (OECD, 2001b). Therefore, this study aimed to assess the acute oral toxicity of the aqueous and methanol extracts of the leaves of *M. tomentosa*.

2. Material and Methods

2.1. Plant material

The *Markhamia tomentosa* leaves were collected during the month of November 2009 and identified by a botanist at the National Herbarium of Cameroon. They were dried at room temperature then pulverized into a powder. The powder obtained was used for the preparation of different extracts.

2.2. Preparation of plant extract

A bio-guided extraction of the *M. tomentosa* leaves powder with few adjustments was done according to the procedure early described by Sosa and collaborators (Sosa *et al.,* 2011).

Three hundred grams of *M. tomentosa* leaves powder were soaked with 500 mL of hot distilled water. After 24 h, the preparation was filtered and evaporated at 45°C in a drying oven to get 26.01 g of crude brown aqueous extract, which yielded 8.67%.

The methanol extract was prepared through a sequential extraction procedure. Five hundred grams (500 g) of leaves powder were macerated sequentially in 1000 mL of hexane, dichloromethane, ethyl acetate and methanol for 72 h and in hot distilled water for 24 h. To estimate the yield at each extraction step, the marc was air-dried and weighed before it was used again. Organic solvents were evaporated under reduced pressure, out of the water which was vaporized in a drying oven at 45°C. The methanol (yield 6.30%) and aqueous extracts (8.67%) which were used in this study were kept at 4°C for the preparation of solutions, administered orally to experimental animals at adequate doses.

2.3. Experimental animals

Healthy female albino Wistar rats between 8 to 10 weeks old (80-120 g) were selected among animals carefully bred under standard conditions (25 ± 2°C with 12 h each of dark and light cycle) in the animal house of the University of Ngaoundere. These rats were fed with a standard commercial diet and drinkable water provided *ad libitum*. Animals were cared and handled in accordance with the internationally recognized standard guidelines and an Ethical clearance was granted by the National Ethical Committee (Reg. N° FWAIRD 0001954).

2.4. Experimental scheme

The acute toxic effects of the aqueous and methanol extracts of *M. tomentosa* leaves were evaluated in accordance with the 423 OECD Guideline method for testing acute toxicity of chemicals (OECD, 2001b). This protocol consists of administration of a single-dose of testing product to preferentially nulliparous and non-pregnant female rat within 14-days (OECD, 2000a). Animals were randomly selected within bred animals, allocated in eight groups of three rats each and kept throughout a week for acclimatization. Treatment of animals was done in two steps. For the first step, three

batches of animals were given orally, distilled water (control group or normal rat), aqueous or methanol extracts of *M. tomentosa* (tested groups). Each test substance was administered sequentially in a single dose of 2000 mg/kg to three rats. Three other batches of animals were treated in the same way as the previous batches. For the second step, aqueous or methanol extracts of *M. tomentosa* (5000 mg/kg) was administrated sequentially to the two remaining groups of animals, as recommended by OCDE protocol.

2.5. Noxious signs exploration

2.5.1. Observation periods

After administration of each test substances, experimental animals were subjected to an observation period of 14 days. During this period, animals were observed individually for the first hour, periodically through the first 24 hours and daily thereafter, till the end of the study period.

2.5.2. Clinical indication sought

Examinations of some clinical signs were done immediately after dosing and during the study observation period. Signs like alertness, visual placing, stereotypy, irritability, reactivity, tremor, convulsions, staggering gait, pupil size, palpebral opening, exophthalmos, salivation, piloerection, arching of the back, wound, nasal discharge, lacrimation, breathlessness, changes in skin and fur were thoroughly checked.

2.5.3. Body weight

The body weight of animal was recorded on the first day before dosing and thereafter on the 7th day and at the last day of the study. This was done each time after three hour of fasting.

2.5.4. Food and water intake

Food and water intake of rats were recorded individually on the day 5th and the 12th day after administration of plant extracts.

2.5.5. Exploratory behaviour

The exploratory behaviour of experimental animals was assessed in an open arena for a 5 min session (Open field test). The test was performed 3 h post dosing, by introducing animals individually and without previous habituation in the centre of a square field (50 × 50 × 30 cm, length × width × height). The floor of this apparatus was divided into 25 small squares of 10 cm^2 each (Micale *et al.*, 2006). The number of crossing (number of line crossed by animal), the appearance and the quantity of stools produced by rats were registered. After each session, the entire inner area of the apparatus was cleaned with alcohol (70°C) and dried to remove odours before the next animal was introduced in the device.

2.5.6. Muscular strength assessment

After the open field test, animals were allow to rest for five minutes and the bar holding test was executed, in order to assess the muscle strength of the animal. Rats were hanged up separately with their forelimbs to a metal bar (60 cm long, 0.5 cm in diameter) horizontally kept at 40 cm above the plinth. The duration of the suspension of the animal only with their forelimbs on the horizontal bar was recorded and the manner to escape (if rat fall off or climb on the horizontal bar) was also took into consideration (Ratnasooriya & Fernando, 2008).

2.5.7. Neuromuscular coordination

Rats were permitted to rest for 30 min and were subjected to a bridge test to evaluate the neuromuscular coordination. For this purpose, animals were placed separately above and in the middle of the metal bar (60 cm long, 0.5 cm in diameter) suspended horizontally at 40 cm above the plinth. The latency and the way (if rats fall off or walk out on the horizontal bar) to quit the bar was noted (Ratnasooriya & Fernando, 2008).

2.5.8. Mortality

During the entire experimental period, each dead animal were subjected to necropsy.

2.5.9. Pathology

All animals were sacrificed at the end of the study and were subjected to *post mortem* examination. All gross pathological changes observed on the skin and orifice or on the surface of majors organs (lungs, liver, kidneys, gastrointestinal tube, heart and spleen) were recorded in other to do the microscopic examination when evidence of any lesion was found.

2.6. Statistical analysis

Values are expressed as mean ± SEM. Statistical differences between control and treated groups were calculated by analysis of variance (ANOVA) followed by Dunnett's test using GraphPad InStat Software 3.0 (USA). P values less than 0.05 were considered significant.

3. Results

3.1. Effects of *Markhamia tomentosa* aqueous and methanol leave extracts on some clinical signs

Treatment of rats with the aqueous or methanol extract at a dose level of 2000 and 5000 mg/kg did not caused any observable adverse signs of intoxication compared to healthy control, 14 days after administration of different extracts.

3.2. Effects of *Markhamia tomentosa* aqueous and methanol leaves extracts (2000 mg/kg) on the body weight gain

Healthy controls animals showed normal growth with 10.43% and 43.65% of body weight gain, recorded during the first and the second week, respectively. Treatment of rats with aqueous or methanol leave extracts (2000 mg/kg) did not significantly altered the growth of animals compared to normal (Figure 1).

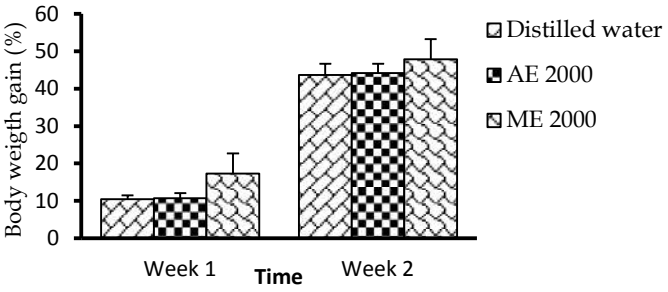

Figure 1: Evolution of the body weight gain of rats treated with the aqueous or methanol leave extracts of *Markhamia tomentosa*. *Data are expressed as mean ± SEM, n = 6. There was no significant difference between healthy control and treated groups. ME 2000 = methanol extract of M. tomentosa at the dose of 2000 mg/kg. AE 2000 = aqueous extract of M. tomentosa at the dose of 2000 mg/kg.*

3.3. Effects of *Markhamia tomentosa* aqueous or methanol leaves extracts (2000 mg/kg) on food and water intake

Treatment of rats with the methanol leaves extract (2000 mg/kg) significantly reduced the water consumption during the first week, but during the second week, this consumption significantly increased compared to the healthy control. Rats treated with aqueous leaves extract (2000 mg/kg) did not exhibit any significant change of water intake during the study. Food intake was not significantly altered during the study period by a single administration of both *M. tomentosa* extracts (2000 mg/kg), as refer to healthy control (Figure 2).

3.4. Effects of *Markhamia tomentosa* aqueous or methanol leaves extracts (2000 mg/kg) on exploratory behaviour, muscle strength and neuromuscular coordination

The number of lines crossed (crossing) by normal rats was 99.60 ± 0.93. Treatment with aqueous leaves extract (2000 mg/kg) resulted in a non-significant increase in the number of crossing (27.51%), compared to the healthy control. Administration of the methanol leaves extract (2000 mg/kg) significantly increase the number of crossing by 100.80% compared to the normal control

(Table 1). The aqueous and methanol leaves extracts did not affect the loose of stools during the observation period of 5 min.

The latency of suspension of normal rats was 12.40 ± 0.58 s. The time of suspension or latency was reduced (26.05%) following treatment of rats with aqueous leaves extract of *M. tomentosa* (2000 mg/kg). The methanol leaves extract (2000 mg/kg) induced a significant reduction of suspension time by 46.21% ($p<0.01$), compared to the normal control. For normal rats and those treated with aqueous leaves extract, the latency corresponded to the time taken for the rat to fall from the bar, whereas those receiving the methanol leaves extract were highly mobile and were able to quickly hoist on the horizontal bar using their legs and tail, thus reducing by this way their suspension time compared to normal (Table 1).

The results of the bridge crossing task show that normal rats were able to cross the bridge from the middle of the bar to one of its ends within 42.80 ± 2.58 s. Rats treated with aqueous or methanol extracts of *M. tomentosa* (2000 mg/kg) exhibited a significant reduction of the time taken to cross the bridge from it middle to one of its extremity by 26.00% or 40.82%, respectively, compared to the normal group (Table 1).

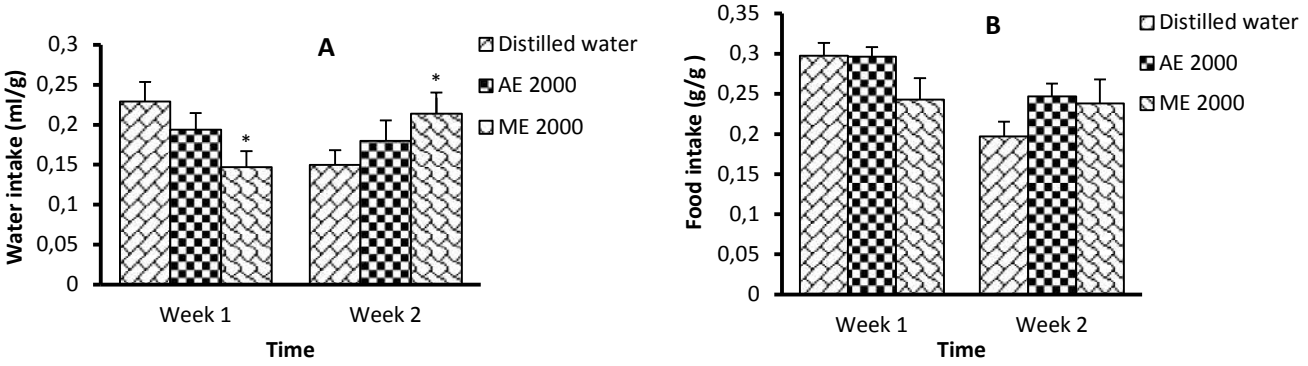

Figure 2: Food and water consumption of rats treated with the aqueous or methanol leave extracts of *Markhamia tomentosa*. *Data are expressed as mean ± SEM, n = 6. * p <0.05 significant difference from the healthy control. ME 2000 = methanol extract of M. tomentosa at the dose of 2000 mg/kg. AE 2000 = Aqueous extract of M. tomentosa at the dose of 2000 mg/kg.*

Table 1: Exploratory activity, muscular strength, and neuromuscular coordination of rats treated with the aqueous or methanol leave extracts of *Markhamia tomentosa*.

Treatments	Open field test		Bar holding test	Bridge test
	Number of crossing	Quantity of stools loosed	Suspension latency (s)	Crossing latency (s)
Distilled water	99.60 ± 0.93	0.00 ±0.00	12.40 ± 0.58	42.80 ± 2.58
AE 2000	127.00 ±4.58	0.00 ±0.00	9.17 ± 1.64	31.67 ± 3.77*
ME 2000	200.00 ± 21.06**	0.00 ±0.00	6.67 ± 0.21**	25.33 ± 2.12**

*Data are expressed as mean ± SEM, n = 6. * p <0.05; ** P <0.01 significant differences compared to the healthy control. ME 2000 = methanol extract of M. tomentosa at the dose of 2000 mg/kg. AE 2000 = aqueous extract at a dose of 2000 mg/kg.*

3.5. Effects of *Markhamia tomentosa* aqueous and methanol leaves extracts (5000 mg/kg) on body weight gain, food and water intake, exploratory behavior, muscle strength, and neuromuscular coordination

When treated with the aqueous or methanol leaves extracts of the *M. tomentosa* at the dose level of 5000 mg/kg, both extracts did not have a similar effect. Regarding the growth of animals treated with the methanol extract, results showed that an increase in body weight gain is associated with a reduction in food and water intake as compared to rats treated with aqueous extract. Results also indicated an increase in the number of crossing, an increase in the latency to cross the bridge, and an increase in the suspension latency compared to rats treated with the aqueous leaves extracts of the *M. tomentosa* (Table 2).

Table 2: Influence of dose 5000 mg/kg of the *Markhamia tomentosa* aqueous or methanol extracts on body weight gain, food and water intake, exploratory activity, muscular strength, and neuromuscular coordination of each rat.

Treatment	Body weight gain		Water intake		Food intake		Open field test		Bar holding test	Bridge test
	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2	Number of crossing	Quantity of stools loosed	Suspension latency (s)	Crossing latency (s)
AE 5000	11.86	35.59	0.19	0.18	0.26	0.20	132.00	0.00	10.00	43.00
	16.67	38.24	0.21	0.20	0.29	0.23	110.00	0.00	7.00	19.00
	7.48	31.78	0.22	0.20	0.30	0.22	153.00	0.00	14.00	36.00
Mean	**12.00**	**35.20**	**0.21**	**0.19**	**0.28**	**0.22**	**131.67**	**0.00**	**10.33**	**32.67**
ME 5000	12.50	51.14	0.17	0.10	0.17	0.17	192.00	0.00	12.00	47.00
	14.61	50.56	0.16	0.10	0.16	0.17	185.00	0.00	24.00	60.00
	15.73	51.69	0.16	0.09	0.16	0.16	131.00	0.00	18.00	54.00
Mean	**14.28**	**51.13**	**0.16**	**0.10**	**0.16**	**0.17**	**169.33**	**0.00**	**18.00**	**53.67**

Data are expressed as individual results, n = 3. ME 5000 = methanol extract of M. tomentosa at the dose of 5000 mg/kg. AE 5000 = aqueous extract at the dose of 5000 mg/kg.

3.6. Mortality and macroscopic lesions related to treatment with *Markhamia tomentosa* aqueous and methanol leaves extracts

Administration of a single daily dose of *M. tomentosa* aqueous or methanol leaves extracts (2000 mg/kg) did not cause any mortality during the study. Autopsy of treated rats did not reveal any injury on the skin, orifices, and mucous membranes or lesions on the surface of some major organs such as lungs, liver, kidneys, gastrointestinal tube, heart, and spleen.

4. Discussion

Medicinal plants have become alternative treatments against many diseases and these practices have increased worldwide and particularly in developing countries, where it was demonstrated that more than 80% of the population rely on phytotherapy for their healthcare (Affy *et al.,* 2018). The biological properties of these medicinal plants are quite interesting for the human being. This is the reason why, great attention should be drawn to the potential toxicity of these bioactive compounds (Mohamed *et al.,* 2011). Therefore, necessary toxicological investigations are useful to ascertain the safety and innocuousness of herbal preparations to valorize their medicinal efficiency. Rats are suitable biological tools for the toxicological evaluation of plant preparations. This is justified by the similarity between harmful effects observed in rats compared to those recorded in human beings (Olson *et al.,* 2000). Studies on conventional lethal dose 50% testing show that female rats are in general slightly more sensitive to toxic activities of active compounds than male (OECD, 2001b).

In this study, a preliminary safety evaluation of the *M. tomentosa* leaves extracts was done through acute toxicity testing. A single dose of the aqueous or the methanol leaves extract of *M. tomentosa* was administered to female rats and these rats were subjected to 14 days observations, according to the protocol of the Organization for Economic Cooperation and Development, which uses pre-defined doses (OECD, 2001b). These methods enable to rank a substance according to the Globally Harmonized System, for the classification of chemicals which cause acute toxicity.

Results obtained from a sighting study performed before this study, with one animal per dose level did not cause death up to the dose of 5000 mg/kg (data not shown). OECD-423 acute toxic class method suggests that a limit test should be conducted when available information suggests that mortality is unlikely at the highest starting dose level which is the dose of 2000 mg/kg in this circumstance (OECD, 2001b). The regulatory guidelines recommended that during acute toxicity challenging observations should include mortality, external changes in physical appearance, changes in respiratory, circulatory, autonomic, and central nervous systems, behavioral patterns, somatomotor activity, and body weight (OECD, 2001b). Clinical check is one of the best ways to assess toxic effects of active compounds on animals because signs and symptoms recorded by the mean of this investigation are directly linked to the systemic toxicity of these active principles on physiological processes (OECD, 2000b).

In this study, rats were challenged with aqueous or methanol leaves extracts of *M. tomentosa* (2000 or 5000 mg/kg). Results showed that no immediate or delay treatment-related undesirable clinical signs were observed for both aqueous and methanol leaves extracts of *M. tomentosa*. These results suggest that these extracts may not have a relevant toxic activity on the biological process.

Healthy conditions could also be monitoring by appreciating the body weight gain (Joshua *et al.*, 2010). It was demonstrated that a slight decrease in the internal organs weights and also in body weight gain could be due to exposure to toxic products and therefore, it may be considered as one of the most sensitive indexes of safety evaluation of new substances (Teo *et al.,* 2002). Our results showed that aqueous or methanol leaves extracts of *M. tomentosa* did not induce any significant change in body weight gain during the observation period. The relation between body weight loss and food and water consumption was demonstrated (OECD, 2001a). However, our results showed a reversible significant decrease in water intake observed during the study in the group of rats treated with methanol extract. This may be due to the taste of this extract or could be assigned to its transitory effect on the autonomic nervous systems since food intake and body weight gain were not significantly affected by treatments (OECD, 2001a). These observations suggest that extracts might be relatively nontoxic.

Adverse side effects of medicinal preparations on the nervous system are not commonly assessed in toxicological studies, although these medicines may be armful on this very important system involved in the coordination of most of the organs of our body. Drugs that cause behavioral alterations, motor dysfunction, stress, depression, anxiety, and memory impairment should not be used despite the good pharmacological properties. Very sensitive and relatively specific tools to assess the effects of natural or synthetic substances on the neuromuscular system are open field, bar holding, and bridge tests (Porsolt *et al.,* 1977). The results of this study demonstrated that methanol extract (2000 mg/kg)

induced a significant increase in rat's locomotor activity to the open field test and reduced the suspension latency to bar holding test. Aqueous or methanol leaves extract of *M. tomentosa* at the dose of 2000 mg/kg significantly reduced compared to control animals the latency to cross the bridge and none of the rats fell off the bridge. It was also displayed that neither aqueous nor methanol leaves extracts did not affect the loose of stools. These findings indicated that all extracts may increase motor and sensory performance as well as muscular strength of animals, suggesting that a dose of 2000 mg/kg, might possess psychostimulant effects (Masoumi-Ardakani *et al.,* 2017). Further studies are needed to understand the mechanisms involved in the activity of our extract at a higher dose on the neuromuscular system.

A complete examination of skin and major internal organs of animals treated with aqueous or methanol did not reveal any visible toxic signs. These results prove the safety of the aqueous or methanol leaves extracts of *M. tomentosa*. Furthermore, no deaths or lethal injury was recorded during this study up to the dose level of 5000 mg/kg. Therefore, the dose that kills 50% of rats (LD_{50}) of the aqueous or methanol would be greater than 5000 mg/kg (OECD, 2001b). Given these findings, these extracts could be labeled unclassified or considered as category 5 products at the globally harmonized system (GHS) of classification of chemical substances and could be considered to be relatively harmless (OECD, 2001b). Altogether, the results of this study suggest that aqueous and methanol leaves extracts of *M. tomentosa* may be safe for animal consumption and could be a very suitable source of potential analgesic drugs since this activity has already been demonstrated (Temdie *et al.,* 2012).

5. Conclusion

In summary, the acute toxicity testing of the *M. tomentosa* aqueous and methanol leaves extracts at the oral doses tested, demonstrated that these extracts did not cause neither any lethality nor adverse changes in the body weight gain and food intake. No treated relative toxic clinical signs were noted. Psychostimulant effects of the methanol extract were observed in treated animals at the dose of 2000 mg/kg and the lethal dose 50% of each extract (LD_{50}) is greater than 2000 mg/kg. Therefore these extracts could be considered as category 5 products in the globally harmonized system of classification of chemical substances and then should be considered to be moderately inoffensive. Consider the doses applied in a pharmacological study there might be a wide margin of safety for the therapeutic use of *M. tomentosa*. Nevertheless, a repeated oral dose toxicity studies are going on to assess the effects of prolonged exposure to *M. tomentosa* leaves extracts.

Authors' contributions: All authors contributed significantly to this study. Temdie GRJ, Kamdem GB, conceptualized this work; Temdie GRJ, Kamdem GB, Minoue KMG, Metchi DMF, conducted investigations, designed methodology, provided resources, and synthesized study data; Temdie GRJ, Minoue KMG, Kada SA, Njiaza J, wrote the initial draft and reviewed the published version under the supervision of Dimo T.

Declaration of interest: The authors certify that they have no affiliation with or financial involvement in any organization with a direct financial interest in the subject matter discussed or materials used in the manuscript.

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