Erratum

Erratum to "Chemical and biological investigations of a toxic plant from Central Africa, Magnistipula butayei subsp. montana" [J. Ethnopharmacol. 103 (2006) 433–438]

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Please note that a new botanical identification of the plant used in our study leads to the conclusion that the plant sample was Dichapetalum stuhlmannii Engler (synonym of D. michelstonii), also known under the vernacular name Umutambasha.

This identification was performed by Professor E. Robbrecht, Head of the Department of Vascular plants, Spermatophytes and Pteridophytes at the National Botanic Garden of Belgium (Meise).

This authentication was confirmed by Dr. Ir. F. J. Breteler (University of Wageningen), one of the specialists of this family.

A voucher specimen correctly identified has been deposited with identification number Karangwa 5108 (BR-S.P. 503344), at the Herbarium of the National Botanic Garden of Belgium.

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Chemical and biological investigations of a toxic plant from Central Africa, *Magnistipula butayei* subsp. *montana*

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Abstract

*Magnistipula butayei* subsp. *montana* (*Chrysobalanaceae*) is known, in the Great Lakes Region, to possess toxicological properties. In this paper, we investigated the acute toxicity (dose levels 50–1600 mg/kg) of its aqueous extract, administered orally to adult *Wistar* rats.

This study demonstrated that the freeze-dried aqueous extract (5%, w/w) possesses high toxicity. The extract caused hypothermia, neurological disorders, including extensor reflex of maximal convulsive induced-seizures at about 2 h after the administered dose, and death occurred (LD\textsubscript{50} = 370 mg/kg) in a dose dependent manner.

Blood parameter evaluation revealed slight variations, but these might not have clinical relevance. Histological examination of internal organs (lungs, liver, heart and kidneys) did not reveal any abnormality in the treated group compared to the control. Therefore, it can be concluded that *Magnistipula butayei* subsp. *montana* aqueous extract, given orally, is toxic and that its target is the central nervous system.

General phytochemical screening revealed that the plant did not contain significant amounts of products known to be toxic, such as alkaloids or cardioactive glycosides, but only catechic tannins, amino acids, saponins and other aphrogen principles in the three parts of the species (fruit, leaf and bark).

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Keywords: *Magnistipula butayei* subsp. *montana*; *Chrysobalanaceae*; Convulsions; Acute toxicity; Hypothermia; Blood parameters

1. Introduction

*Magnistipula butayei* subsp. *montana* (*Hauman*) F. White (*Chrysobalanaceae*) is an evergreen and perennial tropical rainforest tree, which induces severe poisoning of animals and humans in South West Rwanda, North Burundi and East Congo (Desouter, 1991).

According to our ethnobotanical investigations in three Rwandan provinces, people in the Nile-Congo Crest (Cyangugu and Kibuye: 2100–2200 m) use a decoction of tree trunk bark and root, while in the Crest borders (Butare: 1500–1900 m), they widely use the leaf and fruit for killing wild animals (rats, dogs and other predators) and for poisoning humans (Karangwa, 2002). The plant is locally named Umutamasha, Intambasha, Ururamba (Kirundi) (Karangwa, 2002) Umuganza or Umusarwe (Tervuren Museum, Brussels, Belgium). However, its toxicity has not yet been studied; only reports from some ethnobotanists (Troupin, 1978; Desouter, 1991) have related its toxicity.

Indeed, in the Congolese equatorial forest (Central Africa), local people use the tree bark of an other species, *Magnistipula sapini* De Wild., in ordeal poison (Staner and Boutique, 1937).

The present study was initiated to evaluate the toxicity of the aqueous extract from *Magnistipula butayei* subsp. *montana* to substantiate the folklore claims and to detect active principles in order to find an antidote.
2. Materials and methods

2.1. Plant material

Mature tree trunk bark from Magnistipula butayei subsp. montana was collected in Binogo (Rusenyi district, Kibuye province, Rwanda) in August 2001. Binogo is a village located in the mountains of the Nile-Congo Crest near the National Reserve of Nyungwe. The Belgian taxonomist, Georges Troupin (1978), had previously botanically authenticated and deposited some specimens (voucher numbers 15981, 16319, 924, 16035) at the Herbarium of the Institute of Scientific and Technological Research, Butare, Rwanda. Referring to these specimens, we collected and deposited other voucher specimens (Karangwa, 5108) of this plant at the same institute, as well as at the National Botanic Garden of Belgium (Meise) and at the Laboratory of Pharmacognosy, Department of Pharmacy at the University of Liège.

2.2. Preparation of the aqueous extract for biological tests

Mature bark was dried in an air-conditioned room and crushed to obtain a coarse powder (sieve: 1 mm). The extraction was performed by macerating 500 g of crude powder of MBM in 51 distilled water (DW) and percolating through a fresh cotton bed at room temperature for 24 h. The percolate was freeze-dried (Alpha Chriss®) for 36 h and the resulting powder (yield ±5%, w/w) was immediately stored in polyethylene containers.

The lyophilised residue was extemporaneously dissolved in water for biological assays.

2.3. Animal tests

All experimental procedures were carried out in strict accordance with the European Commission Directive (86/609/ EEC) for Guidelines in the Care and Use of Laboratory Animals and were approved by the Ethical Committee for protection of animals at the University of Liège, Belgium.

Adult Wistar rats, weighing between 200 and 300 g, were utilised in various experiments. The animals were all purchased from the Animal Centre of the University of Liège. The animals were kept in an animal room where they were maintained under a controlled temperature (23 ± 1 °C), as well as on a 12 h light/12 h dark cycle, and were provided with food and/or water ad libitum. All treated animals received the Magnistipula butayei subsp. montana aqueous extract (MBMAE) in a single oral dose (expressed as milligrams of the extract per kilogram of rat) by gavage using a feeding needle. Controls received the same solvent.

2.4. Acute toxicity study

The acute toxicity profile was assessed by monitoring convulsions, mortality (determined as LD₅₀ by statistical software Graph Pad Prism, Version 3.0) and other behavioural variations in the same animals, as described in the Hippocratic screening test sheet by Malone and Robichaud (1962). The Hippocratic screening test is commonly used in the preliminary screening of medicinal plants to detect interesting pharmacological activity. Forty-two adult Wistar rats were utilised and grouped into seven groups. Six rats constituted each group. They were deprived of food, but not water, 12 h before starting the experiments. Six groups were respectively treated with six doses (50, 100, 200, 400, 800 and 1600 mg/kg per os (p.o.) of the MBMAE. The one control group received an equal volume of the distilled water vehicle (4 ml/kg).

Observations of toxic symptoms were made and recorded systematically at 1, 2, 4 and 6 h after administration. Finally, the number of survivors was noted after 12 h and these animals were then maintained for a further three days, with observations being made daily. All surviving animals were euthanized with a large dose injection of pentobarbital (100 mg/kg) (Sigma).

2.5. Monitoring of body temperature

Twenty-four adult male Wistar rats were utilised and grouped in four experimental groups. Each group consisted of three treated and three control rats. The treated rats respectively received four doses (50–1600 mg/kg) of the MBMAE and the controls received the vehicle (4 ml/kg). The rectal temperature of each animal was registered immediately before and after administration of an oral single dose of either the extract or the solvent (Table 2).

The probe of a digital thermometer (Ama-Digit®) was introduced into the rectum of the rat to a constant depth of 2.5 cm. Controls served as a reference point for the determination of temperature changes.

2.6. Parameters: blood analysis

The adult male Wistar rats group, fasted overnight, were treated with 800 mg/kg of the MBMAE; the other group (control) received the solvent (4 ml/kg). Two hours later (considered as an elapsed-time of convulsions according to our primary tests), they were anaesthetised for blood collection from a common carotid artery. Blood samples were collected into:

- centrifuge tubes with 2 ml of 20% EDTA for white blood cell (WBC) and red blood cell (RBC) counts and for haemoglobin estimation;
- centrifuge tubes with 3 ml of citrate for coagulating testing;
- heparinised tubes (5 ml) for biochemical determination of electrolytes (Na, K, Cl and Ca), glucose and albumin concentration in rats.

A blood analysis (for both haematology and biochemistry purposes) was carried out within 2 h.

The haematological parameters (total red blood cells and leucocytes (Potron et al., 1990) and haemoglobin (International Committee for Standardization in Haematology, 1978)) were determined by using an autoanalyser (ADVIA 120 Haematology System, Bayer Diagnostics), and coagulation testing (Quick, 1935) by using an automated BCS BEHRING-DADE.
3.1.1. Acute toxicity

Acute toxicity and behavioural observations

(MBMAE) administered to rats provoked convulsive seizures. Results

Statistical analysis

P-values of less than 0.001, 0.01 and 0.05 were respectively considered to be highly significant, very significant and significant.

2.7. Tissue analysis

Two adult male Wistar rats, fasted overnight, were treated with 800 mg/kg of the MBMAE; two controls received the solvent. Two hours later, they were anaesthetised (Nembutal®) and euthanised for tissue studies. Liver, lungs, heart and kidneys were removed and blotted free of blood in buffered formalin.

2.8. Phytochemical screening

Standard screening tests using conventional protocol (Angenot, 1970; Evans, 1996; Wagner and Bladt, 1996) were utilised for detecting the major components (alkaloids, tannins, flavonoids, amino acids, etc.).

2.9. Statistical analysis

The results were expressed as mean ± standard deviation (S.D.). Differences between control and experimental groups were assessed by the Student’s t-test. P-values of less than 0.001, 0.01 and 0.05 were respectively considered to be highly significant, very significant and significant.

3. Results

3.1. Acute toxicity and behavioural observations

3.1.1. Acute toxicity

*Magnistipula butayei* subsp. *montana* aqueous extract (MBMAE) administered to rats provoked convulsive seizures prior to death in a dose dependent manner. The doses of 50 and 100 mg/kg did not induce any visible seizures or death. On the other hand, the doses of 200–1600 mg/kg were lethal and caused death with variable latency from 2 to 72 h (Table 1). The LD$_{50}$ was estimated to be 370 mg/kg body weight (BW). Extract induced an initial arousing and then prolonged reduction of motor activities followed by tonic jerks, followed finally by convulsions. A range of convulsive responses was observed: (i) tonic or clonic events, mainly confined to the head or the head and forelimbs, occasionally with nodding and rearing, but with the animal maintaining its stance (defined herein as a restricted seizure), (ii) tonic or clonic events involving the head and all limbs associated, sometimes with a loss of postural control (defined herein as a generalised seizure). Involvement of the trunk and hindlimbs, therefore, distinguished a restricted from a generalised seizure.

Generalised seizures began in the same way as restricted seizures, with head and forelimb clonic activity, and this was immediately followed by involvement of the trunk and hindlimbs, finishing in the opisthotonus position. Restricted seizures occurred more frequently than generalised seizures. With the doses of 200 and 400 mg/kg, restricted seizures were generally observed, whereas generalised seizures occurred with high doses (800 and 1600 mg/kg). The episode duration of generalised seizures was 30 s–2 min. The intensity and latency of convulsions depended on the administered dose (Table 1).

3.1.2. Behavioural observations

The MBMAE was found to provoke neurological disorders in a dose dependent manner. Besides convulsive seizures and death, the doses of 200–1600 mg/kg BW caused dose-related immobilisation, staring, irregular breathing, tremor, automatisms (bobbing, nodding, chewing), loss of screen grip and righting reflex and convulsive seizures prior to death (Table 1). The intensity and elapsed time of signs and symptoms were respectively dependent on the administered dose. Thus, the small doses of 50 and 100 mg/kg did not show any visible change.

### Table 1

<table>
<thead>
<tr>
<th>Doses</th>
<th>Tonico-clonic convulsive seizures</th>
<th>Mortality</th>
<th>Other toxic symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Intensity (%)</td>
<td>Latency (h)</td>
</tr>
<tr>
<td>DW (control) 4 ml/kg</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MBMAE 50 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MBMAE 100 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MBMAE 200 mg/kg</td>
<td>1/6</td>
<td>+</td>
<td>100 ±3.0</td>
</tr>
<tr>
<td>MBMAE 400 mg/kg</td>
<td>6/6</td>
<td>++</td>
<td>100 ±2.5</td>
</tr>
<tr>
<td>MBMAE 800 mg/kg</td>
<td>6/6</td>
<td>+++</td>
<td>100 ±2.0</td>
</tr>
<tr>
<td>MBMAE 1600 mg/kg</td>
<td>6/6</td>
<td>+++</td>
<td>100 ±1.5</td>
</tr>
</tbody>
</table>

LD$_{50}$ = 370 mg/kg. Imm.: immobilisation; star.: staring; trem.: tremor; LMS: limbic motor seizures (automatisms: bobbing, nodding, chewing; rearing and loss of balance); T/C conv. seiz.: tonico-clonic convulsive seizures; $n$ = ratio of convulsing rats; $n'$ = ratio of dead rats. 0, +, ++, +++: the rating marks, 0: negative; +: weak; ++: strong; +++: very strong; ±: approximate. MBMAE: *Magnistipula butayei* subsp. *montana* aqueous extract; DW: distilled water.
Our experimental pharmacology study demonstrated that the MBMAE caused a significant lowering effect on body temperature in treated rats compared with respective controls. This effect occurred in a dose dependent manner. It was found that MBMAE at a dose of 100 mg/kg BW caused significant hypothermia ($P<0.01$) at 2h following its oral administration (Table 2). This effect was early maximal at a dose of 1600 mg/kg ($P<0.001$), at 1h following its administration and remained for at least 6h (while the animal was still alive).

### 3.3. Haematological and biochemical observations

Haematological and biochemical results are respectively described in Tables 3 and 4.

The haematological values of rats treated with the MBMAE showed a slight significant increase in red blood cells ($P<0.05$) and white blood cells ($P<0.05$) (Table 3). But, there were no significant differences in haemoglobin (HGB) estimation ($P>0.05$) from the controls.

In the blood chemistry analysis, including glucose, albumin, coagulation time and electrolyte determination, no significant changes occurred in the parameters (Table 4). However, there was a significant increase in phosphorus ($P<0.05$) and glucose ($P=0.05$) detection.

### 3.4. Histological evaluation

Organs from rats treated with the MBMAE were macro- and microscopically comparable to the controls. Histopathological examination of tissues (liver, lungs, heart and kidneys) from the Wistar rats indicated that there was no detectable abnormality. No pathological alteration was detected. The architecture of the internal organs was examined and their cellular appearance was comparatively similar in both treated and control groups.

### 3.5. Phytochemical screening

The phytochemical tests revealed that *Magnistipula butayei* subsp. *montana* contained neither cyanogenetic nor cardioactive glycosides nor alkaloids. No significant amounts of terpenes or anthraquinones were detected. However, catechic tannins, leucoanthocyanins, amino acids and saponins (foam value) were found in the three parts of the plant, with a high concentration in leaves (Table 5).
et al., 2004). By contrast, the MBMAE acted by inducing convulsive seizures and lowering body temperature.

However, hypothermia is known to prevent or reduce fever-induced seizures and hyperthermia-induced seizures have been reported in experimental models (McCaughram and Schetcher, 1982; Johnson et al., 1996). We tried here to elucidate the toxicity of a plant, Magnistipula butayeii subsp. montana, traditionally used as a poison in Great Lakes Region for its "inducement of tremor and death". The aqueous extract was used in these tests, since this form is traditionally used by the local population.

The present study has shown that MBMAE elicits immobilisation, staring, tremor, facial and jaw clonus, loss of balance and tonic and/or clonic convulsive seizures prior to death in Wistar rats. The convulsive seizures appeared in a dose-dependent manner, as did death. In addition to the behavioural alterations, hypothermia was also observed. This probably indicates that the aqueous extract contains substances that act upon the neurons of the hypothalamic area involved in body temperature regulation (Bastidas Ramirez et al., 1998; Devi et al., 2003). The loss of screen grip can be taken as an indication of skeletal muscle relaxant activity. The site of action of this activity could be peripheral (at the neuromuscular junction) or central (Kanjianapothi et al., 2004). It is possible that the CNS depression and paralysis of skeletal muscle, which are caused by the MBMAE, tend to modify the response to neuronal disruption and, as a result, hypothermia occurs (Kanjianapothi et al., 2004). Although the hypothermia was statistically significant, it might not have clinical relevance regarding convulsive seizures. Normally, there is a close relationship between seizures and body temperature. High fever frequently induces febrile convulsions in children (Lennox-Buchtal, 1974; Nelson and Ellenberg, 1990) and hyperthermia-induced seizures have been reported in experimental models (McCaughram and Schetcher, 1982; Johnson et al., 1985; Tancerdi et al., 1992; Morimoto et al., 1996; Ahlenius et al., 2002). However, hypothermia is known to prevent or reduce nervous damage (Traynelis and Dingledine, 1988; Maeda et al., 1999; Sanchez-Mateo et al., 2002; Takei et al., 2004; Yager et al., 2004). By contrast, the MBMAE acted by inducing convulsive seizures and lowering body temperature.

Table 5
Phytochemical components

<table>
<thead>
<tr>
<th>Chemical components</th>
<th>Leaf</th>
<th>Fruit</th>
<th>Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catechic tannins</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gallic tannins</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Leucoanthocyanins</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Saponins (foam value)</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Amino-acids</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

The respiratory failure and irregular breathing could also be due to central or peripheral action. Centrally, the irregular breathing could be due to a CNS depression leading to convulsions, whereas, peripherally, the failure could possibly be due to an inhibitory action at the neuromuscular junction. The literature shows that variations in some blood parameters can induce convulsive seizures: hypernatraemia (Türk et al., 2005), hypoglycaemia (Hogan et al., 1985) hypocalcaemia and, hypokalaemia (Astrup et al., 1979; Traynelis and Dingledine, 1988). In our results, haematological and biochemical blood parameters showed values that lacked clinical relevance. The histopathological evaluation failed to reveal any abnormality that would cause convulsive seizures. Nevertheless, behavioural alterations supracritically are good evidence that the MBMAE target is the central nervous system (Mraovitch and Calando, 1999). Therefore, it can be concluded that MBMAE, given orally, is toxic with LD₅₀ = 370 mg/kg and its target is the central nervous system.

The general phytochemical screening revealed that the plant did not contain significant amounts of products known to be toxic, such as alkaloids or cardioactive glycosides, but only catechic tannins, amino acids, saponins and other aphrogen principles in the three parts of the plant (fruit, leaf and bark).

Further toxicological tests are in progress in order to establish the real target, how the toxin acts, and phytochemical studies are being conducted in order to identify the active substance responsible for the toxicity. Non protein amino acids will receive attention because of their possible physiological and toxicological significance (Evans, 1996).

Acknowledgements

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References


