



Acute toxicity studies of the South African medicinal plant *Galenia africana*

Tiza Ng'uni^{a,b}, Jeremy A. Klaasen^{b,*}, Burtram C. Fielding^a

^a Molecular Biology and Virology Laboratory, Department of Medical BioSciences, Faculty of Natural Sciences, University of the Western Cape, Western Cape, South Africa

^b Plant Extract Laboratory, Department of Medical BioSciences, Faculty of Natural Sciences, University of the Western Cape, Western Cape, South Africa

ARTICLE INFO

Keywords:

Galenia africana
Acute toxicity
Skin irritation
Episkin
Dermal sensitization
Local lymph node assay

ABSTRACT

Background: Medicinal plants are used by a large proportion of the global population as complementary and alternative medicines. However, little is known about their toxicity. *G. africana* has been used to treat wounds, coughs and skin diseases and is used in cosmetic formulations such as lotions and shampoos.

Methods: The acute oral and dermal toxicity potential of *G. africana* was analyzed after a single administration of 300 and 2000 mg/kgbw for acute oral toxicity and 2000 mg/kgbw for acute dermal toxicity. Female Sprague-Dawley rats were used for the acute oral toxicity study whereas both male and female Sprague-Dawley rats were used for the acute dermal toxicity study. In the Episkin skin irritation test, the irritation potential of *G. africana* (concentrate) and *G. africana* (in-use dilution) extracts were assessed using the Episkin reconstituted human epidermis. In the dermal sensitization study, female CBA/Ca mice were treated with *G. africana* concentrations of 50, 100 and 200 mg/ml respectively. The vehicle of choice was dimethylformamide which acted as a control.

Results: The results of the acute oral and dermal toxicity studies revealed that the median lethal dosage (LD₅₀) for *G. africana* extract in Sprague-Dawley rats was considered to exceed 2000 mg/kgbw. In the irritation test, the *G. africana* (concentrate) and *G. africana* (in-use dilution) extracts were non-irritant on the Episkin reconstituted human epidermis. In the dermal sensitization study, the stimulation index (SI) values for the mice treated with the *G. africana* extract at concentrations of 50, 100 and 200 mg/ml/kgbw, when compared to the control group, were 1.3, 0.9 and 1.3 respectively. The open application of the extract at the various concentrations did not result in a SI of ≥ 3 in any group. Hence, it did not elicit a hypersensitivity response.

Conclusion: These findings demonstrate that the acute toxicity profile for *G. africana* is acceptable and can subsequently be used for single use in the pharmaceutical and cosmetic industries.

1. Introduction

The use of medicinal plants for various ailments, ranging from minor to chronic, is strongly driven by the increased costs of western medicines, as well as the side effects often linked to the prolonged usage of these medicines [1]. In developing countries the use of medicinal plants has become increasingly important in primary health care, with an estimated 80% of the global population relying on plant-derived medicines for the treatment of various diseases [2]. More recently, natural products have also played a pivotal role in the search for either new drugs or lead compounds used to develop novel therapeutic agents for the treatment of various human diseases [3–5]. Despite the wide use

of traditional herbal medicines, only a few have been tested for their efficacy and safety [6–8]. For this reason, studies looking at the toxicological and pharmacological profile of medicinal plants, their extracts, as well as their formulations have increased dramatically over the last few years [9].

Galenia africana (*G. africana*), commonly known as “kraalbos”, is predominantly found in the Namaqualand, as well as in the Western and Southern Karoo regions of Southern Africa. Traditionally, the plant has been used in the treatment of coughs, wounds, tuberculosis, dermatophytic skin infections, inflammation of the eyes and venereal sores [10–12]. *G. africana* was also used by indigenous tribes of Southern Africa as a component of various cosmetic products such as lotions,

Abbreviations: DPM, disintegrations per minute; ECVAM, European Centre for the Validation of Alternative Methods (ECVAM); *G. africana*, *Galenia africana*; GHS, globally harmonized system; GLP, good laboratory practice; HCA, hexylcinnamaldehyde; LD₅₀, lethal oral dosage; LLNA, local lymph node assay; MTT, methylthiazoldiphenyl-tetrazolium bromide (MTT); NAD, no abnormalities detected; OECD, Organization for Economic Co-operation and Development; PBS, phosphate buffered saline; SI, stimulation index; WHO, World Health Organization

* Corresponding author at: Plant Extract Laboratory, New Life Sciences Building, 4th Floor (Core 1), Department of Medical BioSciences, Faculty of Natural Sciences, University of the Western Cape, Private Bag X17, Bellville 7535, Cape Town, Western Cape, South Africa.

E-mail address: jklaasen@uwc.ac.za (J.A. Klaasen).

<https://doi.org/10.1016/j.toxrep.2018.08.008>

Received 19 July 2017; Received in revised form 6 August 2018; Accepted 12 August 2018

Available online 13 August 2018

2214-7500/© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

decoctions and dressings [11]. More recently, the antifungal [13,14] and anti-mycobacterial [10,15,16] activity of *G. africana* extracts and flavonoid fractions has been reported.

Phytochemical screening of *G. africana* aerial parts have identified various flavonoids as the major secondary metabolites and these include (2S)-5,7,2'-trihydroxyflavanone, (E)-3,2',4'-trihydroxychalcone, (E)-2',4'-dihydroxychalcone and (E)-3,2',4'-trihydroxy-3'-methoxychalcone [10,16]. Flavonoids are a group of plant derived compounds that thought to have numerous properties with significant health benefits. Some of these properties include antibacterial, antiviral, antioxidant, anti-inflammatory and anti-tumor activities [7,17,18]. To date, "kraalbos" has been reported to have some environmental toxicity [19] and *in vitro* immunomodulatory effects [20], but the *in vitro* safety of the plant has not been determined. *G. africana* is being used in the agricultural sector as a biostimulating agent. It is directly applied to field crops, fruit trees and grass in order to increase the polyphenolic, chlorophyll and flavonoid levels. *G. africana* is also used in the cosmetic industry in shampoo, lotion, soap and hand wash formulations. It is for this reason that techniques that increase the concentration of flavonoids should be incorporated in crops and fruits. With the potential of *G. africana* in various agricultural and medical applications the safety of the plant has to be established.

2. Material and methods

2.1. Test material

G. africana was collected from Komaggas farmers, Komaggas (29.7987 S, 17.4825 E), Namaqua District, Northern Cape Province. The formal identification of the plant material used in this study was done by Mr. F. Weitz (Herbarium, Department of Botany, University of the Western Cape, Bellville). Voucher specimens are kept in the UWC herbarium (Vries 1; herb no 6595). The plant was dried for several weeks to maintain the bioactivity and then passed through a hammer mill to produce a powdery material (approximately 2–3 mm). The milled plant material was mixed with 80% ethanol, in the maceration process, to produce a yellow-green to brown-green 20% (w/v) extract with a pH of 6.9. This was then used as a stock solution. This stock solution was used either undiluted or diluted in an appropriate vehicle for the studies described in the following sections. *G. africana* doses and concentrations used in these studies were expressed as the ammonium salt and adjusted for the strength of the stock solution. Chemical analysis of *G. africana* revealed that it contained total solids of 1.7% m/m and a specific gravity of 0.8611. The stock solution was demonstrated to be stable for at least 48 months at ambient room temperature in the dark. Dose formulations were generally prepared on the day of use. A DMA 38 Density Meter (No. 005260) was used to record the *G. africana* density of 0.883 g/ml and this value was used in preparing the dose formulations. Formulation analysis with regard to concentration, homogeneity and stability was done by Charles River Laboratories (Tranent, Edinburg, UK). Furthermore, the plant name has been checked with <http://www.theplantlist.org>.

2.2. Testing facilities and regulatory compliance

The studies described in the following sections were performed by Charles River Laboratories (Tranent, Edinburg, UK). All animal experiments were conducted in the P39 room of the toxicology accommodation at Charles River. Chemical analysis of the extract was performed at Afriplex (Pty) Ltd. All of the studies were performed according to OECD Guidelines for the Testing of Chemicals and Good Laboratory Practices. Study protocols were approved by the testing facilities' Institutional Animal Care and Use Committees. Animal husbandry and use were in accordance with applicable local and international regulations and guidelines. All animals were supplied by Charles River UK Limited, Test Facility Study No. 515938 (Kent, UK) and were

allowed to acclimatise to the toxicology housing at these laboratories for at least 7–8 days before dosing.

2.3. Acute oral toxicity study

The acute oral toxicity test of *G. africana* extract was evaluated in mice according to the procedures outlined by the OECD guideline No. 423 [21]. It was conducted to assess the adverse effects which may result within a short time period following a single oral administration of *G. africana* extract. Oral administration was selected as it is a potential route of accidental human exposure and it allows for hazard classification of test substances. Briefly, nine female (nulliparous and non-pregnant) Sprague-Dawley rats (6–7 weeks old and 165–177 g) were used. Animals were allowed to acclimatise to the toxicology accommodation at the laboratories for at least 14 days before dosing. No formal randomization protocol was followed when allocating them to groups. *G. africana* was administered by oral gavage at doses of 300 and 2000 mg/kgbw to three groups of three female rats. These doses were chosen because no adverse effects were seen after the 300 mg/kgbw dose. Hence a higher dose, 2000 mg/kgbw was administered to assess the effect of the extract. Standard conditions were maintained from the time the animals arrived until the end of the observation period which included an average of temperature 21 °C; average daily humidity of 36% and 83% (minimum and maximum respectively); a 12 h light/dark cycle (7:00-19:00) and minimum ventilation of 15 air changes per hr with standard pellet diet and water. Certificate of analysis for dissolved materials, heavy metals, pesticide residues, pH, nitrates and nitrites were periodically supplied. Dosage and dose volumes of *G. africana* administered to animals are shown in Table 1. Clinical signs were recorded daily, body weights were recorded weekly, and necropsies were performed on day 15. Animals were euthanized by exposure to an increasing concentration of carbon dioxide and major blood vessels were cut to exsanguinate. Necropsy consisted of an examination of the cranial, thoracic and abdominal organs and tissues *in situ*. Carcasses were discarded after this procedure.

2.4. Statistical analysis and electronic data capture software used

No formal statistical analysis was carried out. Test item/control formulation: Dispense version 7.0.3.7 was used to electronically capture the data.

2.5. Acute dermal toxicity study

The acute dermal toxicity test was conducted in accordance with the OECD guideline No. 402 for chemical testing [22,23]. It was conducted to assess the adverse effects that may occur within a short time period following a single dermal administration of *G. africana* extract. Dermal administration was chosen because it is potential route of accidental human exposure and allows hazard classification to be evaluated. Briefly, Sprague Dawley rats, approximately 8 weeks old, comprising 5 males (291–301 g) and 5 females (182–190 g) were used. The standard maintenance conditions of animals were similar to those of conducted in the acute toxicity study. *G. africana* was applied at a dose of 2000 mg/kgbw to sites representing approximately 10% of the total body surface area on the shaved backs of five male and five female

Table 1

Dosage and dose volume of *G. africana* administered to animals in different groups.

Group	Dosage (mg/kgbw)	Dose volume (ml/kgbw)	Animals
1	300	0.34	1–3
2	2000	2.27	4–6
3	2000	2.27	7–9

Table 2
Mean dose volumes of *G. africana* application based on body surface areas.

Sex	Mean dose (ml)	Body surface covered (%)	Dosage (mg/cm ²)
Males	0.78	6	29
Females	0.55	7	20

Sprague-Dawley rats. Application sites were covered with semi-occlusive dressings for 24 h. After the 24 h contact period, the patches were removed, the dosing sites were demarcated and the skin was wiped with water. This was followed by a 14 day observation period. Clinical signs were also recorded daily, body weights were recorded weekly, and necropsies were performed on day 15. Animals were euthanized by exposure to an increasing concentration of carbon dioxide and major blood vessels were cut to exsanguinate. The mean dose volumes applied to the surfaces of the animals are shown in Table 2.

2.6. Statistical analysis and electronic data capture software used

No formal statistical analysis was carried out. Dose formulation: Dispense version 7.0.3.7 was used to electronically capture the data.

2.7. SkinEthic Episkin skin irritation assay

The SkinEthic Episkin irritation assay was conducted in accordance with OECD 439 [24]. This skin irritation assay was used to measure the skin irritation potential of *G. africana* by assessing the cytotoxic effects after a specific exposure period and recovery time. The endpoint of this assay was the estimation of the cell viability by evaluating the ability of the cells to reduce the MTT to its formazan metabolite via mitochondrial reductase. Prior to conducting the irritation assay, *G. africana* was tested to determine if it was capable of reducing (MTT) to its formazan metabolite. 10 µl *G. africana* was added to 2 ml MTT (approximately 0.3 mg/ml) in PBS in a glass universal vial (three replicates) and incubated at 37 °C in a humidified atmosphere with 5% CO₂ for approximately 3 h. Formazan production was assessed by visual inspection. Three replicates of the positive control (eugenol, 10 µl) and negative control (sterile, ultra-pure water, 10 µl) were tested in parallel to demonstrate the efficacy of the MTT solution. Briefly, 10 µl of the concentrated extract (20% (w/v) and the in-use dilution (concentrate diluted to 1% v/v in sterile ultra-pure water) were applied onto the exposed surface of three viable Episkin reconstructed human epidermis units for 15 min. The extract was then washed off and the units placed in an incubator (37 °C in a humidified atmosphere with 5% CO₂) for a recovery period of 42 h. After the recovery period, the skin units were transferred to assay medium containing MTT (0.3 mg/ml) and returned to the incubator for 3 h. Biopsies of the Episkin membranes were then removed and added to isopropanol. The formazan production was assessed by measuring absorbance at 550 nm and the viability of each individual tissue calculated as a percentage of the mean negative control viability. The test substance was considered to be an irritant to the skin if the tissue viability after exposure and post-treatment incubation was less than or equal to 50%. Furthermore, the test substance was considered as “no category” if the tissue viability after exposure and post-treatment incubation was more than 50% (depending on country/regional regulatory requirements) as stipulated by the OECD.

2.8. Dermal sensitization test using the local lymph node assay (LLNA)

The local lymph node assay (LLNA) was conducted in accordance with the OECD test guideline No. 429 [25]. This study examined the delayed contact hypersensitivity or the dermal sensitization potential of *G. africana* in CBA/Ca mice. Briefly, Twenty-two female (nulliparous and non-pregnant) CBA/Ca mice (7–8 weeks old and 16–20 g) were used. Animals were maintained under the following conditions average

daily temperatures of 19–21 °C, average daily relative humidity 54–70%, lighting 12 h light/dark cycle (7:00-19:00) and ventilation minimum of 15 air changes per hr. The same diet and water as that used in the acute dermal and oral toxicity studies was used in this study. *G. africana* concentrations (50 and 100 mg/ml) were prepared by diluting the stock (*G. africana* concentrate) with dimethyl formamide (DMF) (vehicle). *G. africana* (50, 100 and 200 mg/ml) and the vehicle control (DMF) were applied to the dorsal surfaces of both ears (25 µl/ear) of female CBA mice (5 per group) once daily for three days. Hexyl cinnamaldehyde was used as a positive control at concentrations of 5%, 10% and 25%. The findings of a preliminary study showed that 200 mg/ml of *G. africana* caused no significant irritation. All animals were observed daily for signs of toxicity. Treatment sites were scored for irritation on day 3. Body weights were recorded on days 1 (pre-dose) and 6. Three days after the last treatment, animals were administered with 3h-methyl thymidine (20 µCi, iv). After 5 h, the animals were euthanized by exposure to increasing concentrations of carbon dioxide. The major blood vessels were then severed and the auricular lymph nodes were excised. Pooled lymph node cell suspensions from each animal were prepared, washed with phosphate-buffered saline (PBS), and treated overnight with 5% trichloroacetic acid (TCA) at 2–8 °C for approximately 19 h to precipitate DNA. DNA was recovered by centrifugation, resuspended in TCA, transferred to 10 ml scintillation fluid, and measured for radioactivity (Packard 2800TR scintillation counter). Stimulation indices were calculated by dividing the mean dpm (disintegrations per minute) for each *G. africana* treatment group by the mean dpm for the vehicle control group. A stimulation index of 3 or greater was interpreted as evidence that the test substance was a dermal sensitizer. Treatment groups with the vehicle and *G. africana* formulations are shown in Table 3.

3. Results

3.1. Acute oral toxicity study

Results of the acute oral toxicity study are summarized in Table 4. There were no unscheduled deaths among animals receiving *G. africana* extract at either 300 or 2000 mg/kgbw dosage. At necropsy, macroscopic examination on day 15 revealed no abnormalities in any animal. There were no adverse signs of reaction to treatment at a dose of 300 or 2000 mg/kgbw. According to the OECD guidelines, the weight range for female rats is 200–300 g. This range is suggested to provide animals of a size which facilitates the test to be conducted. In addition, the OECD guidelines state that the weight of the animals must fall within an interval of ± 20% of the mean weight of any previously dosed animals. Since the weight of the animals was within the stipulated range, the body weight gain was considered to be acceptable for rats of this age and strain.

3.2. Acute dermal toxicity study

Key findings of the acute dermal toxicity study are summarized in Table 5. There were no unscheduled deaths during the observation period. There were no systemic signs of toxicity recorded in any animal at any observation time point. At necropsy, females on day 15 revealed no macroscopic abnormalities. Among males, enlargement of

Table 3
Treatment of animals with the vehicle and formulations of *G. africana*.

Group	Treatment	Formulation concentration (mg/ml/kgbw)	Animal
1	Dimethylformamide	0	1–5
2	<i>G. africana</i> extract	50	6–10
3	<i>G. africana</i> extract	100	11–15
4	<i>G. africana</i> extract	200	16–20

Table 4
Acute oral toxicity study in Sprague-Dawley rats- key findings.^a

	300 mg/kgbw (group 1)	2000 mg/kgbw (group 2)	2000 mg/kgbw (group 3)
Body weight, g (day 1)	201 ± 4 (3)	204 ± 5 (3)	221 ± 7 (3)
Body weight, g (day 8)	233 ± 10 (3)	240 ± 10 (3)	248 ± 4 (3)
Body weight, g (day 15)	252 ± 6 (3)	260 ± 3 (3)	255 ± 12 (3)
Body weight, gain g (day 1-15)	51	56	35
Daily observations ^b	NAD	NAD	NAD
Necropsy ^b	NAD	NAD	NAD

The body weights of each individual animal were recorded before dosing on Day 1 and on Days 8 and 15.

^a Except for daily observations and necropsy, all values are means ± SD (N).

^b NAD- no abnormalities detected.

Table 5
Acute dermal toxicity study in Sprague-Dawley rats- key findings.^a

	Males 2000 mg/kgbw	Females 2000 mg/kgbw
Body weight, g (day 1)	339 ± 11(5)	239 ± 13(5)
Body weight, g (day 8)	375 ± 21 (5)	251 ± 17 (5)
Body weight, g (day 15)	413 ± 33 (5)	267 ± 19 (5)
Body weight, gain g (day 1-15)	74 ± 30 (5)	28 ± 0 (5)
Daily observations ^b	NAD	NAD
Necropsy ^b	NAD	NAD
Mandibular lymph nodes		
Animal 1	Enlarged (14 × 8 x 2 mm)	
Animal 2	Enlarged (11 × 7 x 2 mm)	
Animal 3	Enlarged (14 × 8 x 2 mm)	
Animal 5	Enlarged (11 × 6 x 2 mm)	
Lungs		
Animal 1	Reddened, all lobes Many dark foci, all lobes (red, pinpoint appearance) Many pale foci, all lobes (cream, ≤ 3 mm diameter)	
Animal 2	Dark focus, left lobe (red, 3 mm diameter)	
Animal 4	3 dark foci, left lobe (red, 1 mm diameter)	
Intestines		
Animal 2	Jejunum- pale Peyer's patch- prominent	
Animal 4	Jejunum-pale, with abnormal (white) contents	
Animal 5	Ileum- distended by contents (6 mm)	
Thymus		
Animal 2	Speckled, right lobe	

^a Except for daily observations and necropsy, all values are means ± SD (N).

^b NAD- no abnormalities detected.

Table 6
Skin irritation study results using the episkin test system.^a

	Treatment group			
	<i>G. africana</i> 20%	<i>G. africana</i> 1%	Negative control	Positive control
Direct MTT	No colour change after 3 h incubation	No colour change after 3 h incubation	No colour change after 3 h incubation	Immediate colour change (dark blue/purple)
Optical density	0.757 ± 0.101	0.831 ± 0.131	0.894 ± 0.033	0.126 ± 0.064
% viability	84% ± 11.26	92.97% ± 14.63	100% ± 3.70	14.10% ± 7.76

^a Except for the direct MTT, all values are means ± SD.

mandibular lymph nodes was recorded in 4 of the 5 animals. Other macroscopic abnormalities included dark and/or pale foci in lungs in 3 males, and intestinal abnormalities such as pale appearance and/or abnormal contents of the jejunum in 2 males. In addition, there were single instances of reddening of the lungs, prominence of Peyer's patch, speckling of the thymus and distension of the ileum. However, these necropsy findings are commonly observed background findings in rats of this strain and are not considered as a result of dermal application of *G. africana* extract. Under the study conditions, the mean lethal oral and dermal dosage (LD₅₀) of *G. africana* extract in Sprague-Dawley rats was considered to exceed 2000 mg/kgbw.

3.3. SkinEthic EpiSkin® skin irritation assay

Key findings of the skin irritation study are summarized in Table 6. The results of the assay were similar for viable Episkin units dosed with the concentrated *G. africana* extract. The *G. africana* concentrate (20% (w/v) resulted in a mean Episkin viability of 84.75% ± 11.26% of the negative control value. The results of the assay were similar for all viable units dosed with *G. africana* (in-use dilution 1% (v/v)). The *G. africana* extract (in-use dilution) resulted in a mean Episkin viability of 92.97% ± 14.63% of the negative control value. Based on these findings, *G. africana* extract (concentrate and in-use dilution) was judged to be non-irritant (no category).

3.4. Dermal sensitization test using the local lymph node assay (LLNA)

Key findings from the local lymph node assay in mice are summarized in Table 7. *G. africana* caused no systemic signs of toxicity. There was no effect of treatment on body weight. Although body weight losses were recorded in 4 of the 20 mice, they were restricted to animals treated with either the 25% or 50% concentrations. There were no body weight losses among control mice or mice treated with undiluted *G. africana* extract. The stimulation indices for mice treated with the *G. africana* extract at concentrations of 25%, 50% and 100% were 1.3, 0.9 and 1.3, respectively. Open application of *G. africana* extract at the given concentrations did not result in a stimulation index of greater than 3 in any group. *G. africana* was considered not to have the potential to cause dermal sensitization.

4. Discussion

The use of natural medicines for the treatment of various ailments has increased over the years. This is attributed to them being considered safer alternatives with less side effects in comparison to synthetic pharmaceuticals [26]. As a result, extensive studies are underway to evaluate the development and use of traditional medicines in advancing their effectiveness and safety [27]. Nonetheless, inadequate scientific data on dosing, quality, toxicity, safety and efficacy of various traditional medicine formulations are available [27–29]. Toxicity testing is vital as it facilitates the evaluation of the possible harmful side effects attributed to these products. Since a number of substances provoke allergic contact dermatitis in humans, assessment of safety by carrying out skin sensitization tests is crucial. Furthermore, it is

Table 7
Stimulation indexes obtained after treatment in the local lymph node assay.^a

Treatment group	Stimulation index
Vehicle control	1
Positive control	
5% HCA	1.7
10% HCA	2.4
25% HCA	5
<i>G. africana</i> 50 mg/ml	1.3
<i>G. africana</i> 100 mg/ml	0.9
<i>G. africana</i> 200 mg/ml	1.3

^a Dimethyl formamide (DMF) was used as the vehicle and hexylcinnamaldehyde (HCA) (5%, 10% and 25%) was used as a positive control.

imperative to evaluate the short or long term effects of these products on human health [26,30].

The studies described in this article were performed to evaluate the toxicity of *G. africana* extract. *G. africana* was virtually non-toxic (lethal dose greater than 2000 mg/kgbw) following single oral administration to female Sprague-Dawley rats. Adverse effects of *G. africana* were assessed by evaluating body weight and through necropsy. All clinical signs, parameters and behaviours were normal with no lesions of internal organs. No signs of toxicity and no mortality were observed. Additionally, *G. africana* was non-toxic (lethal dose greater than 2000 mg/kgbw) following single, 24 h, dermal administration to female and male Sprague-Dawley rats. There were no findings at necropsy that were considered to be related to treatment. Hence, the necropsy findings seen in males are commonly observed background findings in rats of this strain. The concentrated *G. africana* extract as well as the in-use dilution were considered as being non-irritant to the skin. Exposure to the concentrated extract and in-use dilution resulted in episkin viabilities of $84.75\% \pm 11.26\%$ and $92.97\% \pm 14.63\%$ respectively. The negative and positive controls were within the defined acceptance criteria and demonstrated the efficacy of the test system. In the dermal sensitization studies, *G. africana* did not have the potential to cause sensitization. At the time of the study, no data pertaining to the potential of *G. africana* components to cause local irritation in mice, rats or skin was available. The LLNA showed that at the highest (undiluted) concentration, the extract had no toxic effects. In addition, it did not elicit a hypersensitivity reaction in the mice. No concentration resulted in a stimulation index of ≥ 3 in any group. Consequently, it was established that the test item did not have the potential to cause sensitization. These studies were performed in accordance with the OECD guidelines and Principles of Good Laboratory Practice.

Environmental toxicology studies of *G. africana* extracts have been found to have a variety of inhibitory effects (EC_{50}) against *Vibrio fischeri* (0.7 $\mu\text{g/ml}$, 30 min), *Selenastrum capricornatum* (100 $\mu\text{g/ml}$, 72 h), *Daphnia pulex* (30 $\mu\text{g/ml}$, 48 h) and *Poecilia reticulata* (20 $\mu\text{g/ml}$, 96 h) [19]. The minimum inhibitory concentrations for various fungal pathogens (*Alternaria*, *Botrytis*, *Cylindrocarpon*, *Eutypa*, *Fusarium*) of plants showed values ranging from 25 to 100 mg/ml [14] and for *Mycobacterium smegmatis* 0.78 mg/ml [15]. An immune toxicity study of *G. africana* extract found that concentrations of 19 to 500 $\mu\text{g/ml}$ modulate pro-inflammatory cytokines of BALB/c mouse splenocytes [20].

This study investigated the delayed contact hypersensitivity potential, acute oral toxicity, acute dermal toxicity and SkinEthic Episkin *in vitro* irritation assay effects of *G. africana* ethanolic extract. The skin irritation study found it to be a non-irritant and it was shown to be a dermal non-sensitizer in the local lymph node assay in mice. Findings demonstrated that the toxicity profile for *G. africana* is acceptable for its intended use in agricultural and medical applications. All tests were conducted according to the Organization for Economic Co-operation and Development (OECD).

5. Conclusion

The outcomes of this study demonstrated that the ethanolic extract of *G. africana* did not result in any *in vivo* danger. These findings will be included in the documentation for a toxicological review to register *G. africana* extracts as pesticidal adjuvants and plant growth stimulants. Nevertheless, pharmacological and biochemical investigations will be essential in elucidating the mechanism of action and will be beneficial in utilizing this plant as a therapeutic agent. Furthermore, a detailed experimental analysis of the chronic toxicities is important to support these findings. Toxicity assessment of medicinal plants is important in evaluating their safety and sensitizing potential. Clinical trials have not yet been performed on this medicinal plant.

Ethics approval and consent to participate

All studies were conducted to OECD GLP (good laboratory practice) regulations as included in the UK law and under a licence granted by the UK Governmental Department, The Home Office. This document covered all work conducted at the Charles River Laboratories.

Consent for publication

Not applicable.

Availability of data and materials

Additional data, if required, can be made available on request. The plant material listed in manuscript can be shared, if available, on a mutually agreed material transfer agreements.

Conflict of interest

The authors wish to confirm that there are no financial or non-financial conflicts of interest to declare that could have influenced the outcome of this study.

Funding

This project was supported by funds from the Technology Innovation Agency (TIA) of South Africa. Any opinions, findings and conclusions or recommendations expressed in this manuscript are those of the authors and therefore the TIA does not accept any liability in regard thereto.

Authors' contributions

JK was involved in designing the study. JK, BCF and TN compiled and analyzed the results. TN and BCF were involved in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank Charles River International Laboratories, United Kingdom, for performing the acute toxicity studies, skin irritation and the dermal sensitization studies.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2018.08.008>.

References

- [1] M. Leonti, L. Casu, Traditional medicines and globalization: current and future perspectives in ethnopharmacology, *Front. Pharmacol.* 4 (2013) 92.

- [2] World Health Organization WHO, WHO Launches the First Global Strategy on Traditional and Alternative Medicine, [cited 2016 20 July]. Available from: (2002) <http://www.who.int/mediacentre/news/releases/release38/en/>.
- [3] N. Engel, A. Falodun, J. Kuhn, U. Kragl, P. Langer, B. Nebe, Pro-apoptotic and anti-adhesive effects of four African plant extracts on the breast cancer cell line MCF-7, *BMC Complement. Altern. Med.* 14 (2014) 334.
- [4] N. Abu, M.N. Akhtar, S.K. Yeap, K.L. Lim, W.Y. Ho, A.J. Zulfadli, A.R. Omar, M.R. Sulaiman, M.P. Abdullah, N.B. Alitheen, Flavokawain A induces apoptosis in MCF-7 and MDA-MB231 and inhibits the metastatic process in vitro, *PLoS One* 4 (2014) e105244.
- [5] M.N. Hossen, M.Y. Ali, M.H.A. Jahurul, M.M. Abdel-Daim, S.H. Gan, M.I. Khalil, Beneficial roles of honey polyphenols against some human degenerative diseases: a review, *Pharmacol. Rep.* 69 (2017) 1194–1205.
- [6] J.W. Bae, D.H. Kim, W.W. Lee, H.Y. Kim, C.G. Son, Characterizing the human equivalent dose of herbal medicines in animal toxicity studies, *J. Ethnopharmacol.* 162 (2015) 1–6.
- [7] C. Costa, A. Tsatsakis, C. Mamoulakis, M. Teodoro, G. Briguglio, E. Caruso, D. Tsoukalas, D. Margina, E. Dardiotis, D. Kouretas, C. Fenga, C8, Current evidence on the effect of dietary polyphenols intake on chronic diseases, *Food Chem. Toxicol.* 110 (2017) 286–299.
- [8] O.C. Seremet, O.T. Olaru, C.M. Gutu, G.M. Nitulescu, M. Ilie, S. Negres, C.E. Zbarcea, C.N. Purdel, D.A. Spandidos, A.M. Tsatsakis, M.D. Coleman, D.M. Margina, Toxicity of plant extracts containing pyrrolizidine alkaloids using alternative invertebrate models, *Mol. Med. Rep.* 17 (2018) 7757–7763.
- [9] S.M. Dicon, M. Samuthirapandi, A. Govindaraju, P.D. Kasi, Evaluation of *in vitro* and *in vivo* safety profile of the Indian traditional medicinal plant *Grewia tiliaefolia*, *Regul. Toxicol. Pharmacol.* 73 (2015) 241–247.
- [10] S.P. Mativandlela, T. Muthivhi, H. Kikuchi, Y. Oshima, C. Hamilton, A.A. Hussein, M.L. van der Walt, P.J. Houghton, N. Lall, Antimycobacterial flavonoids from the leaf extract of *Galenia africana*, *J. Nat. Prod.* 72 (2009) 2169–2171.
- [11] J.M. Watt, M.G. Breyer-Brandwijk, Medicinal and Poisonous Plants of Southern and Eastern Africa, E & S Livingstone Ltd., Edinburgh & London, 1962.
- [12] J.J.J. De Beer, B.E. Van Wyk, An ethnobotanical survey of the Agter-Hantam, Northern Cape Province, South Africa, *S. Afr. J. Bot.* 77 (2011) 741–754.
- [13] B.C. Fielding, C.L. Knowles, F.A. Vries, J.A. Klaasen, Testing of eight medicinal plant extracts in combination with kresoxim-methyl for integrated control of *Botrytis cinerea* in apples, *Agriculture* 5 (2015) 400–411.
- [14] F.A. Vries, H. El Bitar, I.R. Green, J.A. Klaasen, W.T. Mabusela, B. Bodo, Q. Johnson, An antifungal active extract from the aerial parts of *Galenia africana*, 11th NAPRECA Symposium Book of Proceedings (2005) 123–131.
- [15] S.P. Mativandlela, J.J. Meyer, A.A. Hussein, P.J. Houghton, C.J. Hamilton, N. Lall, Activity against *Mycobacterium smegmatis* and *M. Tuberculosis* by extract of South African medicinal plants, *Phytother. Res.* 22 (2008) 841–845.
- [16] L.A. Ticha, J.A. Klaasen, I.R. Green, S. Naidoo, B. Baker, R.D. Pietersen, Phytochemical and antimicrobial screening of flavanones and chalcones from *Galenia africana* and *Dicrothamnus rhinocerotis*, *Nat. Prod. Commun.* 10 (2015) 1–8.
- [17] E. Middleton Jr, C. Kandaswami, T.C. Theoharides, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer, *Pharmacol. Rev.* 52 (2000) 673–751.
- [18] O.M. Vandeputte, M. Kiendrebeogo, S. Rajaonson, B. Diallo, A. Mol, M. El Jaziri, M. Baucher, Identification of catechin as one of the flavonoids from *Combretum albiflorum* bark extract that reduces the production of quorum-sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1, *Appl. Environ. Microbiol.* 76 (2010) 243–253.
- [19] E. Pool, J.A. Klaasen, Y.P. Shoko, The environmental toxicity of *Dicrothamnus rhinocerotis* and *Galenia africana*, *Afr. J. Biotechnol.* 8 (2009) 4465–4468.
- [20] E. Pool, J.A. Klaasen, Y.P. Shoko, The immunotoxicity of *Dicrothamnus rhinocerotis* and *Galenia africana*, *Afr. J. Biotechnol.* 8 (2009) 3846–3850.
- [21] OECD, Guidelines for Testing of Chemicals: Acute Oral Toxicity-Acute Toxic Class Method, No. 423, (2001), pp. 1–14.
- [22] OECD, Guidelines for Testing of Chemicals, Section 4: Acute Dermal Toxicity, No. 402, Health Effects, OECD Publishing, 1987, pp. 1–7.
- [23] OECD, OECD (Ed.), Guideline For Testing Of Chemicals: Draft Updated Test Guideline 402 on Acute Dermal Toxicity, No 402, 2015, pp. 1–12 Paris.
- [24] OECD, OECD (Ed.), Guidelines For Testing Of Chemicals: *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method, No 439, 2015, pp. 1–21 Paris.
- [25] OECD, OECD (Ed.), Guideline For Testing Of Chemicals: Skin Sensitization: Local Lymph Node Assay, No 429, 2010, pp. 1–20 Paris.
- [26] H. Park, Y.H. Hwang, H.J. Yang, H.K. Kim, K.S. Song, J.Y. Ma, Acute toxicity and genotoxicity study of fermented traditional herb formula Guibi-tang, *J. Ethnopharmacol.* 156 (2014) 182–189.
- [27] G.A. Cordell, M.D. Colvard, Natural products and traditional medicine: turning on a paradigm, *J. Nat. Prod.* 75 (2012) 514–525.
- [28] P. Posadzki, L.K. Watson, A. Alotaibi, E. Ernst, Prevalence of use of complementary and alternative medicine (CAM) by patients/consumers in the UK: systematic review of surveys, *Clin. Med. Lond.* 13 (2013) 126–131.
- [29] S. Ishtiaq, M. Akram, S.H. Kamran, U. Hanif, M.S.K. Afridi, Sajid-ur-Rehman, A. Afzal, A. Asif, M. Younus, S. Akbar, Acute and sub-acute toxicity study of a Pakistani polyherbal formulation, *BMC Complement. Altern. Med.* 17 (2017) 387.
- [30] A. Turon, I. Jordan, C. Bras, A. Soriano, C. Munoz-Almagro, Pneumococcus induced haemolytic-uraemic syndrome and cholecystitis, *An. Pediatr. Barc.* 75 (2011) 351–352.