

# Acute Dermal Toxicity, Cumulative Irritancy, Sub-Chronic Toxicity Test, Delayed Toxicity Assessments and Microbial Quality Analysis of an Herbal Drug as Analgesic

Raphael Nyarkotey Obu, RND, PhD<sup>1\*</sup>, Lawrence Aggrey –Bluwey, BSc, PGDE, MPhil<sup>2</sup>

<sup>1</sup>Nyarkotey College of Holistic Medicine, Tema Community 7, Ghana

<sup>2</sup>Assistant Lecturer, Department of Health Administration and Education, University of Education, Winneba, Ghana

DOI: [10.36348/sijtc.2021.v04i06.006](https://doi.org/10.36348/sijtc.2021.v04i06.006)

| Received: 13.10.2020 | Accepted: 25.10.2020 | Published: 17.06.2021

\*Corresponding author: Raphael Nyarkotey Obu, RND, PhD

## Abstract

This study was design to examine the Acute Dermal Toxicity, cumulative irritancy, Sub-Chronic Toxicity Test, delayed Toxicity Assessments and Microbial Quality Analysis of an Herbal drug for analgesic property as well as the phyto screening potential of the product. Six samples of the product were submitted to the Department of Pharmacology and others for the analysis at the Kwame Nkrumah University of Science and Technology, KNUST, Kumasi, Ghana, as part of the Food and Drug Authority (FDA) herbal drug registration protocol. The result proved that, the herbal product formulated for pain relief is safe to use in experimental animals and therefore could be safe to use as a topical application.

**Keywords:** Pain relief, herbal drug, safety, microbiology, toxicity.

**Copyright © 2021 The Author(s):** This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

## INTRODUCTION

The herbal product formulated as herbal analgesic contains the plant ingredients *Cardiospermum halicacabum*, *senna alexandrina*, and *eucalyptus globulus*. The product was sent for analysis as part of the Food and Drug Authority (FDA), Ghana, requirement for herbal product registration as stipulated in the Public Health ACT, 2012(ACT 851).

### **Eucalyptus globulus**

Research suggests that eucalyptus oil eases joint pain. In fact, many popular over-the-counter creams and ointments used to soothe pain from conditions like osteoarthritis and rheumatoid arthritis contain this essential oil. Eucalyptus oil helps to reduce pain and inflammation associated with many conditions. A 2013 study by Jun et al.[3] also concluded that, inhalation of eucalyptus oil was effective in decreasing patient's pain and blood pressure suggesting that eucalyptus oil inhalation may be a nursing intervention for the relief of pain.

### **Cardiospermum halicacabum**

A 2006 study conducted by Venkatesh and Krishnakumari [5] shows that the plant exhibits the anti-inflammatory properties, justifying its use in rheumatoid arthritis treatment. A more recent study in 2019 by Kandasamy et al. [1] with the aim to evaluate the effectiveness of *Cardiospermum halicacabum* Leaves Soup on Chronic Knee found positive association with the plant and pain relieving effect.

### **Senna alexandrina**

A 2018 study conducted by Elansary et al. [2] demonstrates that *Senna alexandrina* exhibits potential on stomach pain.

## METHODOLOGY

Three tests were conducted at the Kwame Nkrumah University of Science and Technology, KNUST, Kumasi, Ghana, on the product. The tests will be analyze here per department

**Department of Pharmaceutics****Table-1: Microbial Quality Analysis**

Test	Results	Specification (BP 2015)
Total Aerobic viable Count (NA 37C)	1.0*10 <sup>4</sup> cfu/g	<5.0*10 <sup>5</sup> cfu/g
Total Yeast/Moulds (SB: 25C, 5days)	1.0*10 <sup>3</sup> cfu/ml	<5.0*10 <sup>4</sup> cfu/g
Enterobacteria (LTB; 37C,48h)	7.1*10cfu/ml	<1.0*10 <sup>4</sup> cfu/g
Escherichia coli (MaC;37°C; 48h)	Not detected	Absent (in 1g)
Salmonella (BSA; 37°C;48h)	Not detected	Absent (in 25g)

**REMARKS**

The total microbial load of Pain Relief was within the acceptable limits (BP 2015; category C of herbal products) there were no pathogenic

microorganisms present. The herbal ointment has complied with the BP specifications for microbiological quality.

**Department of Herbal Medicine****Table-2: Phytochemical and physicochemical studies**

<b>1.ORGANOLEPTIC PROPERTIES</b>	
Form	- Semi-solid
Colour	- Light green
Taste	- Characteristic
Odour	- Minty
<b>2.PHYSCOCHEMICAL PROPERTIES</b>	
pH (1% Aqueous extract)	- 6.76
Solubility	- Insoluble in water - Partially soluble in ethanol and - Methanol; soluble in petroleum ether and diethylether.
Washability	- Easily washed with soap
<b>3.PHYTOCHEMICAL PROPERTIES</b>	
Reducing sugars	- Positive
Saponins	- Negative
Alkaloids	- Negative
Flavonoids	- Negative
Phytosterols	- Positive
Terpenoids	- Positive
Tannins	- Positive
<b>4.TLC CHROMATOGRAPHIC PROFILE</b>	
Stationary phase	- Pre-coated silica gel plates
Mobile Phase	- Chloroform: Pet-ther (9:1)
Sample used	- Chloroform extract
Detecting reagent	- Anisaldehyde
Results	- Five (5) spots were observed after spraying and gently warming. - four (4) yellow spots (Rf's:0.95, 0.81, 0.68, 0.30) - One (1) pink spots (Rf's; 0.74)

**Department of Pharmacology**

TEST CONDUCTED: Acute Dermal Toxicity, cumulative irritancy, Sub-Chronic Toxicity Test and delayed Toxicity Assessments.

**Acute Dermal Toxicity Test**

Group A1: 1 G OF Pain Relief was applied uniformly as possible over the shaved or clipped area on quinea pigs. It was held in contact with the skin with a porous gauze dressing and nonirritating tape (a patch) to ensure that the animals could not ingest the ointment throughout the period. After 24 hours of the exposure, the residual test substance was removed and the test sites were scored. The sites were further observed for a period of 7 days for delayed acute toxicity symptoms. Group A2 was the non-drug control group: 1.0g petroleum jelly was applied to the shaved areas.

**Cumulative Irritancy Test**

Group B1: 0.2g quantities of Pain Relief were applied as in group A2, but this time, twice daily for 14 days. The residual test substance after each day however was not removed until after the day 14. To group B2, 0.4g was applied. Patches were removed daily and sites scored prior to next patch application. Group B3 was control group in which 0.4g of petroleum jelly was applied.

**Sub-chronic Toxicity Test**

With 0.2g per patch. Pain relief was applied twice daily for 28days in group C1 and 0.4g per patch in group C2 (The ointment was applied at the same test sites daily). Residual test substances after each day however were removed. Test sites were scored prior to next patch application. Group C3 was a control group in which 0.4g of petroleum jelly was applied.

**Table-3: Results: Acute Dermal Toxicity, cumulative irritancy, Sub-Chronic Toxicity Test And delayed Toxicity Assessments**

Groups	Test	Score
A1	Acute Dermal Toxicity	0
A2	Acute Dermal Toxicity – control (vehicle)	0
B1	Cumulative Skin Irritancy (0.2g)	0
B2	Cumulative Skin Irritancy (0.4g)	1
B2	Cumulative Skin Irritancy – control	0
C1	Sub-chronic Toxicity (0.2g)	0
C2	Sub-chronic Toxicity (0.4g)	1
C3	Sub-chronic Toxicity – control	0

**Scale for scoring**

0= no evidence of any effect

1= minimal, faint, uniform, or spotty erythema

2= pink-red erythema covering most or all of the contact site

3= pink-red erythema visibly uniform in entire contact site

4= bright red erythema with or without petechiae or papules

5= deep red erythema with or without vesiculation or weeping

Accompanying edema (swelling) is recorded with an ‘e’ and is described as mild, moderate or severe as compared with the normal surface of the surrounding skin. If/when a reaction of 4 or 5 is observed, test discontinued and the score attained will be entered for the balance of 28 days.

Acute dermal toxicity testing showed no evidence of any effect compared with the vehicle treated qiuinea pigs’ i.e. no primary irritation, rash, desquamation, pruritus, corrosion and sensitization. There was no delayed acute toxicity reaction. A 14-day cumulative skin irritancy test no showed minimal, faint, uniform or spotty erythema only after using twice the intended quantity to be used. A 28-day sub chronic toxicity assessment also revealed no evidence of toxic effect. There were no primary irritations, corrosion, ulceration and necrosis. There was no loss of autonomic reflexes, sedation, behavioral changes and respiratory depression and mortality.

Observation were not limited to evaluation of skin and fur, and eyes and mucous membranes, but also respiratory and circulatory effects, autonomic effects (such as salivation) central nervous system effects (including tremors and convulsions), changes in the level of activity, gait and posture, reactivity to handling or sensor stimuli, altered strength and stereotypies or bizarre behavior (e.g. Self-mutilation, walking backwards). Pain relief is safe to use in experimental animals and therefore could be safe to use as a topical application

**CONCLUSION**

The herbal product formulated for pain relief is safe and can be use in humans. This is due to the fact, the Observation during the study periods were not limited to evaluation of skin and fur, and eyes and mucous membranes, but also respiratory and circulatory effects, autonomic effects (such as salivation) central nervous system effects (including tremors and convulsions), changes in the level of activity, gait and posture, reactivity to handling or sensor stimuli, altered strength and stereotypies or bizarre behavior (e.g. Self-mutilation, walking backwards). Pain relief is safe to use in experimental animals and therefore could be safe to use as a topical application

**ACKNOWLEDGMENT**

To the investigators at KNUST, Kumasi, Ghana, who conducted the tests on the herbal product.

**REFERENCES**

1. Kandasamy, M., Manju, G., Manimegalai, N., Abhilash, V., & Prathima, P. (2019). At What Extent Does *Cardiospermum Halicacabum* relieves chronic Joint Pain among Elderly? An Experiment in Urban community of Tamilnadu, India. *Indian Journal of Public Health Research & Development*, 10(5), 173-177.
2. Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., Ali, H. M., Elshikh, M. S., Abdel-Salam, E. M., El-Esawi, M., & El-Ansary, D. O. (2018). Bioactivities of Traditional Medicinal Plants in Alexandria. *Evidence-based complementary and alternative medicine: eCAM*, 2018, 1463579. <https://doi.org/10.1155/2018/1463579>
3. Jun, Y. S., Kang, P., Min, S. S., Lee, J. M., Kim, H. K., & Seol, G. H. (2013). Effect of eucalyptus oil inhalation on pain and inflammatory responses after total knee replacement: a randomized clinical trial. *Evidence-based complementary and alternative medicine: eCAM*, 2013, 502727. <https://doi.org/10.1155/2013/502727>
4. KC, V. B., & Krishnakumari, S. (2006). *Cardiospermum halicacabum* suppresses the production of TNF-alpha and nitric oxide by human peripheral blood mononuclear cells. *African Journal of Biomedical Research*, 9(2), 95-99.