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Acute oral toxicity, analgesic effect and healing activity of the root's extracts of *Strophanthus gratus* (Wall. & Hook.) Baill

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Abstract
The plant is native to west and central Africa. It is cultivated for its medical and toxic applications in Ivory Coast. The seeds are rich in strophantine and its aqueous extract is a strong poison. The plant is used for the treatment of heart disease. The root decoction is used to heal wounds and appears to be a powerful aphrodisiac. A phytochemical screening achieved on this aqueous and ethanolic extracts of *Strophantus gratus* simply unveiled the presence of polyphenols, flavonoids, free quinones, tannins, alkaloids, anthocyanins and saponins. Acute toxicity test provides evidence that AESG and EESG are safe up to dose level of 3000mg/kg bw. AESG and EESG have shown analgesic activity similar to Naproxen which suppresses pain sensitivity (acetic acid-induced writhing test). Both AESG_50% and EESG_50% ointment formulations revealed wound healing activity by optimizing healing conditions after a cut and stitches.

Keywords: *Strophantus gratus*, phytochemical screening, AESG, EESG, acute toxicity, wound

1. Introduction
Strophantus gratus is a woody shrub up to 25 m in length and giving off a transparent or translucent latex. Sap, wood, fruits and seeds go into preparations of arrow poisons for example the sap of the fresh bark is mixed with that of *Parquetina nigrescens* (Periplocaceae) to produce arrow poison in Congo. However, the root bark is used as an antidote for food poisoning. In Ivory Coast, the aqueous extract of the leaves is used to treat constipation and to lower fever [1]. In West Africa, a paste from the roots is applied to wounds, including guinea worm sores. The plant is used as a good luck charm [2]. Despite the extensive use of *Strophantus gratus* by African traditional healers. There is very little scientific data on this Ivorian plant in the literature. All this motivated the work which consisted in studying the acute oral toxicity, analgesic effect and healing activity aqueous and ethanolic root extracts of *Strophantus gratus*.

2. Materials et Methods
2.1 Plant Material (Purchase, Identification and Extraction)
The roots of *Strophantus gratus* were purchased from a traditional therapist in Abatta (Abidjan, Ivory Coast), It was identified and authenticated by the botanists of Jardin Botanique du centre National de Floristique under the specimen (10939 CNF).
The roots were pulverized using a Nima electric grinder. The brown powder roots of *Strophantus gratus* weighed, divided into two parts, a first part was cold macerated in distilled water for 48 hours with stirring. The second part was macerated with ethanol as solvent under the same conditions as the first. Using the rotary evaporator, the solvents were removed by evaporation. The lyophilizer have allow to remove solvent residues to obtain dry powders [3]. Finally, the dried crude aqueous and ethanolic extracts were used for the experiments. AESG = Aqueous root extract of *Strophantus gratus* and EESG = Ethanolic root extract of *Strophantus gratus*.

2.2 Animal material
Adult albino rats of both sexes were obtained from the University pet store. Tests on rats

provide data regarding efficacy and safety. Rodents share 90% of their genes with humans. These animals can, for example, suffer from obesity, stress or other diseases which are observed in humans. All good laboratory procedures were observed.

2.3 Phytochemical characterizations

AESG and EESG were subject to the identification by precipitation reaction and staining according to the methodology of the phytochemical screening [4].

Table 1: Phytochemical characterizations

Phytocompounds	Reagent of identification	Indicator (positive reaction)
Anthraquinones	NH_4OH	Yellow color
Anthocyanin	H_2SO_4 and NH_4OH	Black color
Terpenoids	$CHCl_3$, H_2SO_4	Brown color
Polyphenols	$FeCl_3$ (2%)	Dark blue or greenish color
Flavonoids	Hydrochloric alcohol, Magnesium shavings and Iso-amyl alcohol	Pink-orange or purplish color
Catechic tannins	Formalin and HCl	Gelatinous precipitate
Gallic tannins	Sodium acetate and $FeCl_3$	Blue-black color
Free quinones	NH_4OH	Red to purple color
Saponosides	Foam index	Persistent foam
Alkaloids	$HgCl_2$ and KI (Mayer)	Reddish-brown precipitate
	Picric acid (Hager) I_2 and KI (Wagner)	Creamy-white precipitate
Coumarins	KOH and HCl	Trouble or precipitate
Sterols and polyterpenes	Acetic anhydride acid and H_2SO_4	Color from purple to blue or green
Mucilage	Absolute ethanol	Flocculent precipitate
Volatile oils	NaOH and HCl	Black color
Cardiac glycosides	$CHCl_3$, H_2SO_4	Brown color

2.4 *In vitro* acute toxicity study

The acute toxicity study was carried out according to the Organization for Economic Cooperation and Development (OECD) [5]. The rats were divided into 5 groups of 6. It was randomly selected and scored for individual identification. Grp 1: a control group (normal saline solution), Grp 2: treated group (300 mg/kg bw), Grp 3: treated group (900 mg/kg bw), Grp 4: treated group (1500 mg/kg bw), Grp 5: treated group (3000 mg/kg bw). The animals were observed almost constantly for changes in behavior, appearance and mortality.

2.5 *In vivo* analgesic effect

The method consisted of detecting the peripheral analgesic activity of the extracts and was carried out by dividing by random group of rats. Acetic acid was injected into all groups of rats 60 minutes immediately after the rats received; Grp 1: negative control group (10 mL/kg of Distilled water), Grp 2: positive control group (125 mg/kg bw of Naproxen), Grp 3: treated group (65 mg/kg bw of AESG), Grp 4: treated group (85 mg/kg bw of AESG), Grp 5: treated group (105 mg/kg bw of AESG), Grp 6: treated group (125 mg/kg bw of AESG), Grp 7: treated group (65 mg/kg bw of EESG), Grp 8 : treated group (85 mg/kg bw of EESG), Grp 9: treated group (105 mg/kg bw of EESG), Grp 10: treated group (125 mg/kg bw of EESG). The number of writhes in each treated group was compared with the control group and the percent reduction of writhes count was calculated as follows [6].

$$\text{Percent reduction of writhes (\%)} = \frac{\text{NGRPcontrol} - \text{NGRPtreated}}{\text{NGRPcontrol}} \times 100$$

with NGRPcontrol = the mean number of writhes for negative control group
and NGRPtreated = the mean number of writhes for treated group

2.6 In vivo Healing activity

To assess healing activity of aqueous and ethanolic root extracts of *Strophantus gratus* (AESG and EESG), standard models of excisional and incisional wounds were used.

2.6.1 Excision wound

An area was marked on the depilated back of the rat by a standard ring, and an excision wound was inflicted by cutting away a circular piece of 350 mm^2 full thickness of skin on the back of the rat. The animals were divided into 3 groups of 6: Grp 1: placebo control group = simple ointment (Shea butter), Grp 2: treated group (50% ointment of the aqueous root extract of *Strophantus gratus* = AESG_50%), Grp 3: treated group (50% ointment of the ethanolic root extract of *Strophantus gratus* = EESG_50%). The progressive changes in wound area were monitored and noted every other day for 16 days. Wound contraction was calculated as percentage of the reduction in wounded area as given in the formula below [7]:

$$\text{Percentage of wound contraction} = \frac{\text{wound 0} - \text{wound n}}{\text{wound 0}} \times 100$$

with: wound 0 = wounded area at day (0),
wound n = wounded area at particular day (n)

2.6.1Ointment's formulation

A simple ointment (shea butter) and two different doses (AESG_50% and EESG_50%) of medicated ointments of *Strophantus gratus* were prepared according to formula described in Ivorian Pharmacopoeia (Table 2). Shea butter, AESG, EESG, and Calcium benzoate (preservative = E 213) were whipped until homogeny, creamy and smooth. they were stored in airtight jars at room temperature [8].

Table 2: Preparation of the various ointments

Ingredients	Placebo Shea butter	Ointment AESG_50%	Ointment EESG_50%
AESG (g)	0	27.75	0
EESG (g)	0	0	27.75
Shea butter (g)	55.5	27.75	27.75
Calcium benzoate (g) (E 213)	1.5	1.5	1.5
Total (g)	57	57	57

2.6.2 Incision wound

Under the same conditions as for the previous model, a longitudinal paravertebral incision of 3.5 cm in length was made and sutured by 1 cm this time. The animals were divided into 3 groups of 6. Grp 1: placebo control group (Simple ointment = Shea butter + E 213), Grp 2: treated group (AESG_50% ointment), and Grp 3: treated group (EESG_50% ointment). The sutures were removed on day 8 post-incision and the treatment was continued. Then, the tensile strength was measured on the 10th day and calculated using weight technique [9]:

$$\text{Percentage of tensile strength} = \frac{\text{tensile strength exp} - \text{tensile strength ctrl}}{\text{tensile strength ctrl}} \times 100$$

with tensile strength ctrl = the mean tensile strength of placebo control group

and tensile strength exp = the mean the tensile strength of treated group

2.7 Statistical analysis

The analysis of variance was used to compare the averages between more than two groups. Values with $p < 0.05$ were considered statistically significant. Graphs were obtained using the Microsoft Excel 2016 spreadsheet. Statistical analyzes were performed in GraphPad Prism for Windows.

3. Results

3.1 Yield of extracts

The percentage yield of AESG (aqueous root extract of *Strophantus gratus*) and EESG **(**ethanolic root extract of *Strophantus gratus*) was presented in Table 3.

Table 3: Yield (%) of ethanolic and aqueous root extracts of *Strophantus gratus*

Extract	Mass (g)	Yield (%)
EESG	4.75	9.50
EASG	4.05	8.10

3.2 Phytochemical analysis

Phytochemical screening of AESG and EESG was presented in Table 4.

Table 4: Results of phytochemicals analysis

Secondary metabolites	EESG	EASG
Cardiac glycosides	Absence	Absence
Coumarins	Absence	Absence
Mucilages	Absence	Absence
Volatile oils	Absence	Absence
Anthraquinones	Absence	Absence
Sterols and polyterpenes	Absence	Absence
Terpenoids	Absence	Absence
Alkaloids	Presence	Presence
Anthocyanins	Presence	Presence
Catechic tannins	Presence	Presence
Gallic tannins	Presence	Presence
Free quinones	Presence	Presence
Saponins	Presence	Presence
Polyphenols	Presence	Presence
Flavonoids	Presence	Presence

3.3 Acute toxicity study

The acute oral toxicity study shows that AESG and EESG are safe at the 3000 mg/kg bw dose because no animal died and no behavioral changes and appearance were observed during two weeks. Therefore, the LD_{50} of aqueous and ethanolic root extracts of *Strophantus gratus* is greater than 3000 mg/kg bw.

3.4 Analgesic effect

The result of the analgesic effects AESG and EESG is presented in Table 5 and Figure 1. AESG and EESG showed significant analgesic activity in reducing number writhing induced by acetic acid.

Table 5: Analgesic effects of AESG and EESG

Groups	Treatment	Dose (mL/kg or mg/kg bw)	Writhing frequency	Percent inhibition (%)
Grp 1	Distilled water	10	85.50±1.53	0
Grp 2	Naproxen	125	10.38±1.71	87.86
Grp 3	AESG	65	50.67±1.29	40.74
Grp 4	AESG	85	31.45±1.24	63.22
Grp 5	AESG	105	15.21±1.13	82.21
Grp 6	AESG	125	08.15±1.46	90.47
Grp 7	EESG	65	55.15±1.83	35.50
Grp 8	EESG	85	45.85±1.63	46.37
Grp 9	EESG	105	21.12±1.93	75.30
Grp 10	EESG	125	10.10±1.13	88.19

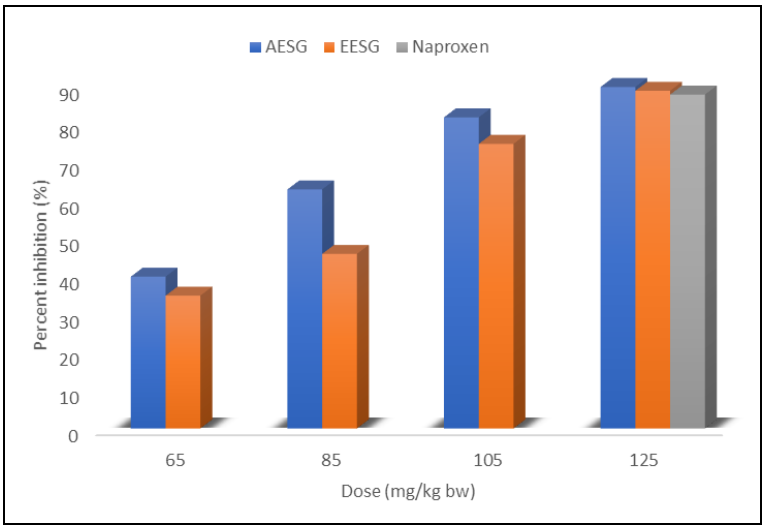

Fig 1: Comparison between AESG, EESG and Naproxen on analgesic effects

3.5 Healing activity

3.5.1 Excision wound

AESG_50% and EESG_50% ointments showed important wound contraction against control placebo (simple ointment). The study confirmed that considerably shorter healing time was recorded by EESG_50% and AESG_50% ointments against simple ointment, (see Table 6 and Figure 2).

Table 6: Wound-healing effect of Simple ointment, AESG_50% and EESG_50% ointments in excision wound model

Wound area (mm^2) on post-wounding day and (% wound contraction)						
Group: ointment	**Day (0)**	**Day (2)**	**Day (4)**	**Day (8)**	**Day (12)**	**Day (16)**
Grp 1: Control placebo	350.00±1.16	301.79±1.74 (13.77%)	250.43±1.66 (28.45)	209.57±1.58 (40.12%)	180.28±1.56 (48.49%)	106.56±1.28 (69.55%)
Grp 2: AESG_50%	350.00±1.38	254.98±1.76 (27.15%)	151.46±1.81 (56.72%)	50.24±1.36 (85.71%)	00±00 (100%)	00±00 (100%)
Grp 3: EESG_50%	350.00±1.22	251.65±1.12 (28.10%)	149.87±1.17 (57.18%)	35.08±1.66 (89.98%)	00±00 (100%)	00±00 (100%)

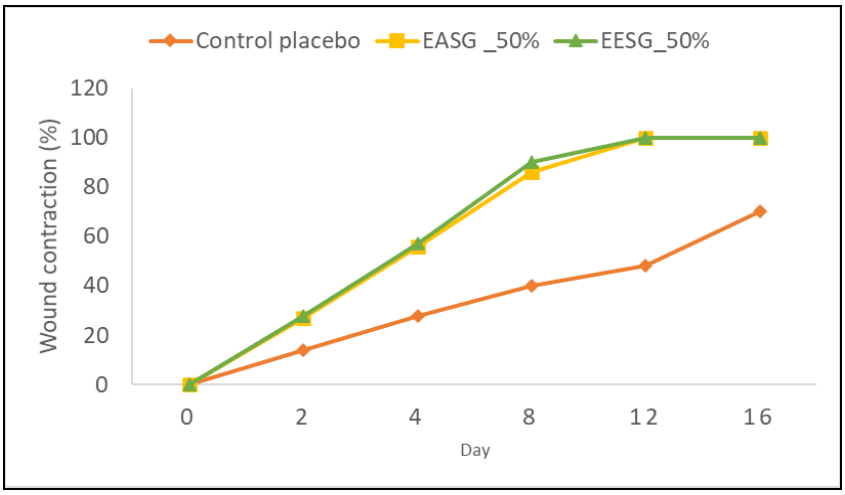

Fig 2: Comparison between control placebo (simple ointment), AESG_50% and EESG_50% ointments on wound contraction effects (wound-healing).

Period of Epithelialization

Epithelialization period is faster with AESG_50% and EESG_50% ointments compared to simple ointment (see Table 7).

Table 7: Effects of control placebo ointment, AESG_50% and EESG_50% ointments on period of epithelialization

Groups	Epithelialization (day)
Grp 1: Control placebo = Simple ointment	19.52±1.48
Grp 2: AESG_50% ointment	09.75±1.36
Grp 3: EESG_50% ointment	10.05±1.03

3.5.2 Incision wound

AESG_50% and EESG_50% ointments were effective in increasing breaking strength as compared with Control placebo (simple ointment) as shown in Table 8.

Table 8: Percentage of tensile strength of Control placebo (simple ointment), AESG_50% and EESG_50% ointments

	Wound breaking strength (g)	**Tensile strength (%)**
Grp 1: Control placebo	279.87±1.49	0
Grp 2: AESG_50%	497.51±1.13	77.58
Grp 3: EESG_50%	512.62±1.27	83.16

4. Discussion

The phytochemical characterization of aqueous and ethanolic extracts of *Strophantus gratus* revealed the presence of major phytochemical groups such as flavonoids, tannins, alkaloids, anthocyanins, polyphenols, saponins and free quinones. Alkaloids have several pharmacological activities which are: analgesic, anticholinergic, anti-malaria (quinine), antihypertensive, anti-tumor (taxol and vincristine), sympathomimetic, cardio-depressive, anesthetic, narcotic and diuretic properties [10]. Flavonoids possess several interesting biological activities such as antimicrobial, antifungal, anti-inflammatory activity and activity against lipid peroxidation and hematologic damage [11]. The terpenoids and tannins are promoted wound-healing process [12].

According to the study from the acute oral toxicity test, the LD_{50} was estimated to be greater than 3000 mg/kg in rats. Aqueous and ethanolic root extracts of *Strophantus gratus* (AESG and EESG) can be used in the treatment of several diseases without danger [9].

The peripheral analgesic action of AESG and EESG at a dose of 125 mg/kg bw is similar to that of Naproxen at a dose of 125 mg/kg bw. The reference product Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) often used to treat pain and injury. NSAID work by inhibiting an enzyme important in the mechanism of inflammation and pain (COX or cyclooxygenase) [13].

Pain is not a disease; it is a warning signal sent from an area of the body that is undergoing aggression or dysfunction. This signal is transmitted by nerves from the affected area to the brain, which processes and interprets the pain message. An analgesic relieves or eliminates pain by blocking the pain signal that is transmitted to the brain. It therefore does not address the cause of the assault, or the dysfunction. Some analgesics also have an action against fever [15,16].

The healing of a wound is an extremely complex phenomenon, specific to each organism. It involves the processes of repairing a localized lesion and regenerating tissue. The physiological mechanisms of this process involve many cell types (fibroblasts and epithelial cells), successive cascades of intracellular messengers and molecules involved in the general anabolism of the organism (GAG, fibronectin, collagen, etc.). These phenomena are regulated by extracellular matrix factors and inflammatory cells. Three successive overlapping phases make up the healing process: first a vascular and inflammatory phase. Then, a budding phase corresponding to the proliferative phase with the development of the granulation tissue. This tissue helps to fill the loss of substance. Indeed, a new tissue is created thanks to neo angiogenesis and cell proliferation. The budding phase continues until epithelialization or re-epimerization is obtained. Finally, the later phase of scar remodeling [17]. The capacity of the wound to regenerate remains subject to many constraints. The speed and quality of healing are dependent on the general state of health of the individual, the etiology of the lesion, the condition and location of the wound and whether or not it occurs infection. Therefore, disruption of any of these phases can result in delayed or complicated wound healing, and hence in a chronic wound [18].

5. Conclusion

The study of AESG (aqueous root extract of *Strophantus gratus*) and EESG (ethanolic root extract of *Strophantus gratus*) provides evidence that extract is safe up to dose level of 3000 mg/kg bw. AESG and EESG have analgesic effects which suppress sensitivity to pain. AESG_50% and EESG_50% ointments are designed to help heal small scarring, optimize healing conditions after a cut, surgery, stitches, cosmetic surgery or even an accident.

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