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EFFECT OF AN AQUEOUS EXTRACT OF *SACOGLOTTIS GABONENSIS* STEM BARK ON BLOOD PARAMETERS DURING BURN-INDUCED WOUND HEALING IN WISTAR RAT

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ABSTRACT

Buruli ulcer is a chronic, debilitating skin infection caused by *Mycobacterium ulcerans*. In Côte d'Ivoire, traditional treatment is oral and cutaneous with *Sacoglottis gabonensis*. This study was to investigate the effect of *Sacoglottis gabonensis* total aqueous stem bark extract (TAESg) on blood parameters during burn-induced wound healing in Wistar rat for 35 days. 24 rats were divided into four group of six rats. Group 1 received no treatment; groups 2, 3 and 4, treated orally and dermally, respectively, received distilled water; *Flukocin*[®] 500mg at 14.28mg/kg bw and *Baneocin*[®] 10 g at 81.6mg/kg bw; and TAESg at 3.5 and 5000mg/kg bw and TAESg at 3.5 and 5000mg/kg b.w. Three blood samples were taken, i.e. before induction, after induction and after wound treatment. These blood samples were used to determine the levels of certain blood parameters. This study showed a reduction in thrombocyte count and sedimentation rate and restoration of leukocyte, albumin and CRP levels in TAESg-treated rats compared with post-treatment group 2 rats. The same observations were made in group 3 rats treated with *Flukocin*[®] and *Baneocin*[®]. In conclusion, TAESg possesses healing activity by acting on key biological markers of the wound healing process. It would be interesting to evaluate the haemostatic and anti-inflammatory effects of this extract in rats.

KEYWORDS

Sacoglottis gabonensis, Buruli ulcer, Wounds, Healing, Biomarkers and Rat.

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INTRODUCTION

African populations are faced with the emergence or re-emergence of infectious diseases, the treatment and monitoring of which represent an additional socio-economic problem¹. Among these pathologies is Buruli ulcer (BU), a cutaneous

infection caused by a mycobacteria, *Mycobacterium ulcerans* (*M. ulcerans*), present in the environment^{2,3}. Africa is the most affected region, particularly West Africa where it is highly endemic with a prevalence of 50 %, the majority of cases being in children under 15 years of age⁴. Côte d'Ivoire is one of the most endemic countries, with over 2,000 new cases per year⁵. In 2007, the National Buruli Ulcer Control Program (PNLUB) counted a cumulative total of 25,617 cases between 1978 and 2006⁶. In 2008, the WHO reported nearly 30,000 cases in Côte d'Ivoire⁷. Antibiotic therapy is used to treat UB at all stages of the disease for 4 to 8 weeks, resulting in healing of early lesions, stabilization of the disease or regression of lesions to allow less debilitating surgical removal³. At present, there is no effective long-term vaccine against UB⁸. Numerous side effects of this antibiotic therapy have been reported, especially in children, including liver, kidney and hearing damage, and atrophy of limb muscles⁹.

Surgical wound treatment, whether or not combined with antibiotic therapy, offers satisfactory results, but also has other constraints, including high cost and long hospitalization and/or treatment times for many patients, mostly from rural areas^{10,9}. This long duration is due to the fact that wound healing goes through several processes enabling reconstitution of the necrotic skin¹¹. To combat and help reduce the use of surgery in the management of BU, it makes sense to turn to herbal treatments. Indeed, many patients often turn to traditional medicine and more specifically medicinal plants, as a first-line treatment for this pathology¹². With this in mind, the World Health Organization (WHO) has recommended a strategy based on the accessibility, efficacy, safety and quality of herbal medicines¹. In Côte d'Ivoire, *Sacoglottis gabonensis* is a plant used orally and dermally to treat Buruli ulcer¹³. With a view to verifying the efficacy of this plant on wound healing and recommending it as an alternative treatment for Buruli ulcer in Côte d'Ivoire, we set out to evaluate the effect of the total aqueous extract of *Sacoglottis gabonensis* stem bark (TAESg) on blood parameters during the healing of

burn-induced wounds in rats. Specifically, the effect of TAESg on the wound healing process will be assessed by changes in hematological and biochemical parameters.

MATERIAL AND METHODS

Plant material

It consists of the stem bark of *Sacoglottis gabonensis* (Baille) Urban (Humiriaceae). These barks were collected in March 2021 at Ingrakon in the Alepe region, a town located about 45 km from the Abidjan district. A sample was identified in accordance with the one kept at the National Floristic Center (CNF) under number 1154 dated June 16, 1965.

Animal material

The experiments were conducted on male and female albino rats of the *Rattus norvegicus* species of Wistar strain. They all come from the animal house of the Laboratory of Physiology, Pharmacology and Pharmacopoeia (L3P) of the University Nangui Abrogoua (UNA). They are 3 to 4 months old, and their body weight ranged from 140 to 200g. They were housed in plastic cages with stainless steel lids and fitted with feeding bottles. A layer of wood shavings was placed at the bottom of the cages to provide bedding.

The animals were kept at a temperature of $22 \pm 2^{\circ}\text{C}$ with a 12-hour photoperiod. Rats were fed daily with IVOGRAIN® pellets (Côte d'Ivoire) and tap water without discontinuity in feeding bottles. The experimental protocol and animal handling procedures were carried out in accordance with good laboratory practice¹⁴.

Technical Equipment

The entire apparatus consisted of a grinder (Retsch SM 100, Hann, Germany) to finely powder the stem bark of *Sacoglottis gabonensis* a Denver S-234 electronic balance (Belgium) for weighing; a Selecta® oven (Spain) to dry the extracts; an Ovan MCG05E magnetic stirrer (Europe) to homogenize the extracts; a razor to clip the fur; butane gas topped with a hot plate to heat the plate; a 3cm diameter metal cylinder topped with a rod for wound induction; and a thermocouple (HANNA, HI

93530, Italy) to measure the cylinder temperature. For the measurement of the various parameters, dry tubes, purple containing EDTA and black containing sodium citrate, were used for blood sampling. a centrifuge (HERAEUS SEPATECH, Germany) for obtaining plasma; an automated counter analyzer (Sysmex XT 2000i, Japan) for the dosage of hematological parameters; a Semi-automated (Prietest Touch Robonik, India) and a biochemistry automaton (Cobas C111, Switzerland) for the dosage of biochemical parameters. The basic equipment consisted of a funnel, absorbent cotton, and Wattman No. 1 filter paper for filtering the decoction; a 500mL graduated cylinder for measuring water quantities; a gastric tube for force-feeding the animals; cotton wool, gauze pads, and non-irritating adhesive tape for dressing the wounds.

Chemical Substances and Pharmacodynamics

The chemical substances and reagents consisted of diethyl ether (Cooper) for anesthetizing the animals; 90% alcohol for skin disinfection; *Flukocin*[®] 500mg capsule (medopharm pvt.ltd, India) and *Baneocin*[®] 10g powder (Sandoz GmbH, Austria) as reference substances; and a Cobas kit for CRP measurement.

METHODS

TAESg preparation

Freshly harvested bark is crushed into small pieces and then dried in the laboratory on the bench at 25°C for four weeks. The dried bark is ground to a fine powder using a Retsch SM 100 mill. The total aqueous extract is prepared according to the method described by Kouassi¹⁵. Four hundred grams (400g) of *Sacoglottis gabonensis* stem bark powder is dissolved in two liters (2L) of distilled water and boiled for 30 minutes. After cooling, the decoctate is filtered, first on absorbent cotton, then on Wattman N°1 paper. The filtrates are oven-dried at 50°C for 48 hours. A dry brown powder, TAESg, is obtained. This powder, which will be used to prepare the various concentrations of TAESg, is stored in the refrigerator at -5°C until the days of manipulation.

Evaluating healing activity

Healing activity has been evaluated in an experimental burn model in rats^{16,17}. The adaptation was in terms of temperature and wound induction time, in order to create extensive wounds¹⁸. Twenty-four (24) rats were evenly divided into four groups of six, with three male and three female rats separated into individual cages.

Burn induction

Rats were anesthetized by inhalation in a bell containing cotton soaked in ethyl ether for 30 seconds to 1 minute. The dorsal flank of anesthetized rats was shaved and cleaned with alcohol, 24 h before burn induction. Experimental burns were induced using a 3cm diameter metal cylinder connected to a rod with a handle in all rats of the different groups except group 1. The cylinder, heated to a temperature of 200°C, was applied for 20 seconds, pressing lightly on the surface of the rats' shaved skin to induce extensive deep second-degree burns¹⁸. This burn is characterized by epidermal and dermal damage, with phlyctenes (a liquid of vascular origin forming a bubble that develops at the epidermal-dermal interface) with a red background and whitish areas¹⁹.

Animal treatment

All animals in these different groups received a volume of 1mL/100g b.w. by cannula gavage once a day from 9 a.m. over a period of 35 days. These animals were then treated orally and dermally as follows:

- Group 1, uninduced, receives no treatment;
- Group 2, the induced negative control, received distilled water by gavage and local application;
- Group 3, the positive induced control, received *Flukocin*[®] 500mg (medopharm pvt.ltd, India) at a dose of 14.28mg/kg bw orally and *Baneocin*[®] 10g (Sandoz GmbH, Austria) at a dose of 81.6mg/kg bw dermally;
- Group 4, the induced test, received TAESg at 3.5mg/kg bw orally and 5000mg/kg bw dermally.

Biological blood test

Blood samples were taken according to the method described by Abdullahi *et al*²⁰. In this study, three

blood samples were taken, before wound induction, four days after wound induction and at the end of the experiment. Blood taken immediately was collected in tubes containing the anticoagulant ethylene diamine tetra-acetic acid (EDTA) for blood count (CBC)^{21,22} using an automatic analyzer (Sysmex XT-2000 i, Japan), in black tube containing sodium citrate for sedimentation rate and in dry tubes for C-Reactive Protein (CRP)²³ using an automated biochemistry system (Cobas C 111, Switzerland).

Statistical analysis

Data are analyzed using Graph Pad Prism 8.0.1 (San Diego, CA, USA). Results are expressed as the mean followed by the standard error of the mean ($M \pm SEM$). Statistical significance is determined by ANOVA 1 followed by the Turkey test. These tests will give us the degree of significance for $p < 0.05$. In the presentation of results, symbols (*, **, ***, ****/ #, ##, ###, ####) will indicate significant decreases and increases compared with controls.

RESULTS AND DISCUSSION

Changes in sedimentation rate during wound healing

Before induction, the sedimentation rate in rats from groups 2, 3 and 4 treated respectively with distilled water, reference substances and TAESg, was statistically identical ($p > 0.05$) to that of rats from group 1, the healthy control untreated at both 1st and 2nd hour (Table No.1).

On day 4 after wound induction, a very highly significant increase ($p < 0.0001$) was observed in all the different groups 2, 3 and 4 compared with group 1, the healthy control. The same observation was made in rats from groups 3 and 4 compared with group 2, the negative control.

After 35 days of treatment, the sedimentation rate still showed a highly significant increase ($p < 0.0001$) in rats from group 2 treated with distilled water, while those from groups 3 and 4 treated respectively with the reference substances and the extract, showed a significant increase ($p < 0.01$) compared with group 1, the untreated healthy control. However, the sedimentation rate was

significantly reduced in rats from groups 3 and 4 treated respectively with the reference substances and the extract, compared with group 2, the negative control treated with distilled water.

Table No.1 Values are presented as Mean followed by Standard Error on the Mean ($M \pm ESM$). The comparison is between group 1 and groups 2, 3 and 4 on the one hand and between group 2 and groups 3 and 4 on the other. $p < 0.05$, $n = 6$;

Group 1: untreated healthy controls;

Group 2: negative controls treated with distilled water orally and dermally;

Group 3: positive controls treated with *Flukocin*[®] 500 mg at a dose of 14.28mg/kg bw. c. and with *Baneocin*[®] 10g at a dose of 81.6mg/kg bw by the oral and cutaneous routes respectively;

Group 4: Test treated with TAESg at a dose of 3.5mg/kg bw by the oral route and at a dose of 5000mg/kg bw by the cutaneous route; *: represents decreases and #: represents increases.

Hematological and biochemical parameters during wound healing

Before wound induction, analysis of the results showed that the leukocyte, thrombocyte, albumin and CRP levels of groups 2, 3 and 4 treated with distilled water, reference substances and TAESg respectively, were statistically identical ($p > 0.05$) to those of rats in group 1, the untreated healthy control (Table No.2).

After wound induction, the leukocyte, thrombocyte and CRP levels of groups 2, 3 and 4 treated with distilled water, reference substances and TAESg respectively, showed a highly significant drop compared with group 1, the untreated healthy control (Table No.3). On the other hand, albumin levels in rats from groups 2, 3 and 4 treated with distilled water, reference substances and TAESg respectively, showed a highly significant drop compared with group 1, the healthy control.

After 35 days of treatment, rats in groups 2 and 3 treated with reference substances and TAESg respectively, experienced a highly significant drop in leukocytes, thrombocytes and CRP levels, and a highly significant increase in albumin levels, compared with the negative control group 2 treated

with distilled water (Table No.4). However, compared with group 1, the untreated healthy control, the levels of these different parameters were statistically identical in rats from groups 3 and 4 treated respectively with the reference substances and TAESg, with the exception of thrombocytes, where a highly significant increase was observed. As for group 2, the positive control treated with distilled water, leukocyte, thrombocyte and CRP levels remained high, while albumin levels remained low compared with group 1, the untreated healthy control.

Table No.2 Values are presented as Mean followed by Standard Error on the Mean ($M \pm ESM$). The comparison is between group 1 and groups 2, 3 and 4 on the one hand, and between group 2 and groups 3 and 4 on the other. $p < 0.05$, $n = 6$;

Group 1: untreated healthy controls;

Group 2: negative controls treated with distilled water orally and dermally;

Group 3: positive controls treated with *Flukocin*[®] 500 mg at a dose of 14.28mg/kg bw. c. and with *Baneocin*[®] 10 g at a dose of 81.6mg/kg bw by the oral and cutaneous routes respectively;

Group 4: Test treated with TAESg at a dose of 3.5mg/kg bw by the oral route and at a dose of 5000 mg/kg bw by the cutaneous route; *: represents decreases and #: represents increases.

Table No.3 Values are presented as Mean followed by Standard Error on the Mean ($M \pm ESM$). The comparison is between group 1 and groups 2, 3 and 4 on the one hand, and between group 2 and groups 3 and 4 on the other. $p < 0.05$, $n = 6$;

Group 1: untreated healthy controls;

Group 2: negative controls treated with distilled water orally and dermally;

Group 3: positive controls treated with *Flukocin*[®] 500mg at a dose of 14.28mg/kg bw. c. and with *Baneocin*[®] 10g at a dose of 81.6mg/kg bw by the oral and cutaneous routes respectively;

Group 4: Test treated with TAESg at a dose of 3.5 mg/kg bw by the oral route and at a dose of 5000mg/kg bw by the cutaneous route; *: represents decreases and #: represents increases.

Table No.4 Values are presented as Mean followed by Standard Error on the Mean ($M \pm ESM$). The comparison is between group 1 and groups 2, 3 and 4 on the one hand, and between group 2 and groups 3 and 4 on the other. $p < 0.05$, $n = 6$;

Group 1: untreated healthy controls;

Group 2: negative controls treated with distilled water orally and dermally;

Group 3: positive controls treated with *Flukocin*[®] 500mg at a dose of 14.28mg/kg bw. c. and with *Baneocin*[®] 10g at a dose of 81.6 mg/kg bw by the oral and cutaneous routes respectively;

Group 4: Test treated with TAESg at a dose of 3.5 mg/kg bw by the oral route and at a dose of 5000mg/kg bw by the cutaneous route; *: represents decreases and #: represents increases.

DISCUSSION

Wound healing is a natural biological process that takes place after the soft tissues of the body have been opened by a hot mechanical, chemical, electrical or infectious agent, with the aim of healing the lesion by filling in lost substances and reuniting the wound edges^{24,25}.

Hematologically, before induction, quantitative results showed no significant variation in leukocyte count, thrombocyte count or sedimentation rate in all rats of the different batches. However, after induction, leukocyte count, thrombocyte count and sedimentation rate showed a highly significant increase in rats from groups 2, 3 and 4 compared with group 1. In fact, leukocytes are primarily responsible for the body's immune defense in the event of aggression²⁶. Thrombocytes are involved in hemostasis and play a role in local vasoconstriction²⁷. Thus, wound induction would have triggered an inflammatory reaction resulting in the active release of leukocytes, thrombocytes and activation of factor XII with proteolysis cascades leading to the production of fibrin from fibrinogen, triggering coagulation²⁸. This increase in fibrinogen could be one of the causes of the rise in both sedimentation rate and thrombocyte count.

However, after treatment, the leukocyte levels of rats in groups 3 and 4 treated respectively with the

reference substances and with TAESg showed a highly significant drop compared with those of rats in the positive control group. In fact, this reduction in leukocyte levels in group 3 rats was due to the synergistic effect of *Flukocin*[®] and *Baneocin*[®], which are antibiotics designed to combat microbial infection. The same applies to rats in group 4, as TAESg contains an isocoumarin, bergenin, which is said to have antimicrobial activity²⁹. This would explain the drop in leukocyte levels. The drop in sedimentation rate and thrombocyte count in TAESg-treated group 4 rats is thought to be due to the presence of tannins in TAESg. Tannins have healing properties, strengthening blood vessels and contributing to the accumulation of vitamin K in the body³⁰⁻³¹. Similarly, in group 3 treated with *Flukocin*[®] and *Baneocin*[®], this reduction was due to the combined action of *Flucloxacillin*, *Bacitracin* and *Neomycin*. These substances are used clinically to heal wounds, burns and ulcers. However, any substance with healing power is a hemostatic. Inflammatory cells such as leukocytes and thrombocytes promote the migration and proliferation of endothelial cells, leading to neo-vascularization of connective tissue, which synthesizes extracellular matrices, including collagen, resulting in re-epithelialization of the skin³².

These results are similar to those obtained by Singh *et al*³³, who showed that aqueous extract of *Pleurotus ostreatus* at doses of 100 and 300mg/kg bw restored platelet count and sedimentation rate.

Biochemically, the results showed a highly significant fall in albumin levels and a highly significant rise in CRP levels in rats from groups 2, 3 and 4 treated with distilled water, reference substances and TAESg respectively, compared with group 1, the untreated healthy control, after wound induction. The drop in albumin levels could be due to the heat induced during wound induction. In fact, heat causes protein denaturation, which is responsible for the loss of skin elasticity and hence inflammatory activity³⁴. Protein denaturation is mainly characterized by changes in hydrophobicity, electrostatics, hydrogen bonds and disulfide

bridges, which stabilize molecules^{34,35}. As for CRP, its level increased in a highly significant way, as the heat induced by the causal agent during induction leading to the loss of skin continuity would be at the origin of an inflammatory stimulation responsible for the secretion of IL-6 by macrophages and T cells responsible for the release of CRP in the liver^{36,37}.

After treatment, rats in groups 3 and 4 treated respectively with the reference substances and TAESg experienced a restoration of albumin and CRP levels compared with rats in the untreated healthy control group. This restoration of the different albumin and CRP levels of TAESg-treated rats in group 4 is explained by the presence of several phytochemicals in TAESg. These phytochemical compounds, such as flavonoids, tannins, bergenin, polyphenols and saponosides, are involved in biological activities during the wound-healing process through interaction with various cells, namely leukocytes, thrombocytes, endothelial cells and fibroblasts³⁸. In fact, these phytochemicals in the extract are thought to shorten the inflammatory phase, leading more rapidly to the epithelialization phase and thus accelerating wound healing. According to Sudsai³⁹, flavonoids increase the migration of fibroblasts, the cells responsible for contractile activity in wounds. Tannins have hemostatic activity, precipitating and protecting proteins against protein denaturation⁴⁰. In group 3 rats treated with reference substances, the restoration of these parameters was due to *Flucloxacillin*, *Bacitracin* and *Neomycin*, used clinically as anti-infectives, which stopped all inflammatory activity. Epithelialization of wounds treated with the extract was faster than that of negative controls treated with distilled water, but identical to that of positive control rats treated with the reference substances. This rapid epithelialization could be due to the presence of tannins and flavonoids, which, by activating fibroblasts, promote keranocyte proliferation and migration. This was demonstrated by⁴¹ during work on *Spathodea campanulata* (Bignoniaceae) in wound treatment.

The persistence of high levels of leukocytes, thrombocytes and CRP, including low levels of albumin and sedimentation rate, in the distilled water-treated rats of the positive control group would be explained by an infectious state of the wounds. This infectious state would translate into chronic inflammation due to the absence of adequate treatment, resulting in prolonged protein denaturation and high liver activity, leading to non-healing of the wounds.

Table No.1: Changes in sedimentation rate before, after induction and after treatment

S.No			Group 1	Group 2	Group 3	Group 4
1	Before induction	1h	6.2 ± 0.15	5.8 ± 0.39	6.2 ± 0.20	7.2 ± 0.11
		2h	12.3 ± 0.31	14.2 ± 0.18	13.8 ± 0.21	12.9 ± 0.33
2	4 days after induction	1h	7.2 ± 2.66	21.2 ± 0.58####	19.7 ± 1.64####	20.2 ± 1.72####
		2h	15.4 ± 1.50	33.2 ± 2.80####	34.5 ± 2.48####	34.4 ± 2.72####
3	35 days after treatment	1h	7.6 ± 2.80	17.4 ± 2.55####	9.8 ± 2.56####	9.7 ± 2.69####
		2h	14.3 ± 1.48	29.9 ± 0.52####	18.8 ± 1.63####	19.6 ± 0.76####

Table No.2: Variation in levels of some biological parameters before wound induction

	Leucocytes (10 ³ /mm ³)	Thrombocytes (10 ³ /mm ³)	Albumine (Alb))	C-Reactive Protein (CRP)
Group 1	10.38 ± 0.63	358.9 ± 40.68	3.39 ± 0.19	0.020 ± 0.006
Group 2	10.09 ± 0.69	388.0 ± 30.44	3.60 ± 0.16	0.023 ± 0.003
Group 3	9.98 ± 0.73	417.0 ± 8.74	3.33 ± 0.13	0.023 ± 0.004
Group 4	9.86 ± 0.77	400.8 ± 7.67	3.61 ± 0.06	0.015 ± 0.004

Table No.3: Variation in levels of some biological parameters after wound induction

	Leucocytes (10 ³ /mm ³)	Thrombocytes (10 ³ /mm ³)	Albumine (Alb))	C-Reactive Protein (CRP)
Group 1	10.04±0.54	375.6 ± 18.37	3.35 ± 0.16	0.016 ± 0.004
Group 2	16.12±0.17####	1007 ± 136.5####	2.43 ± 0.16***	0.135 ± 0.004####
Group 3	16.80±0.13####	1016 ± 129.7####	245 ± 0.17***	0.170 ± 0.008####
Group 4	15.98±0.19####	1040 ± 131.1####	2.33 ± 0.14***	0.198 ± 0.004####

Table No.4: Variation in levels of some biological parameters after wound treatment

	Leucocytes (10 ³ /mm ³)	Thrombocytes (10 ³ /mm ³)	Albumine (Alb))	C-Reactive Protein (CRP)
Group 1	10.78 ± 0.38	389.1 ± 30.32	3.25 ± 0.23	0.013 ± 0.003
Group 2	15.58 ± 0.29####	994.7 ± 124.1####	1.99 ± 0.29****	0.132 ± 0.005####
Group 3	10.36 ± 0.41****	630.14 ± 31.1#### ****	3.28 ± 0.23####	0.022 ± 0.004****
Group 4	10.20 ± 0.22****	622.68 ± 20.78#### ****	3.16 ± 0.21####	0.027 ± 0.004****

CONCLUSION

At the end of the study, it was found that daily oral administration of *Sacoglottis gabonensis* total aqueous stem bark extract to extensive second-degree burn wounds in rats, combined with dermal administration, resulted in wound healing for 35 days. TAESg possesses healing activity by acting on key biological markers of the wound healing process that promote wound closure. The effect of TAESg via the two associated pathways is similar to that of the combination of *Flukocin*[®] and *Baneocin*[®]. This study has enabled us to verify and confirm scientifically the traditional use of this plant in the treatment of Buruli ulcer in Côte d'Ivoire.

However, more in-depth studies of this extract need to be carried out on haemostasis and inflammation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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