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Anti-nociceptive and anti-inflammatory activities of ethanol stem bark extract of *Odina barteri* in experimental animals

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Abstract

Medicinal plants have been a cornerstone of traditional medicine for centuries, offering a rich source of bioactive compounds with therapeutic potential. This study investigated the analgesic and anti-inflammatory effect of the ethanol stem back extract of *Odina barteri* in rodents. Analgesic effects of the extract was determined mice using acetic acid-induced writhing and formalin induced paw oedema in rats. The anti-inflammatory activity was assessed using xylene induced ear oedema in mice and egg- albumin- induced inflammation in rats respectively. The acetic acid and formalin induced pain methods showed dose dependent analgesic effects. There was a significant ($p<0.05$ and $p<0.01$) reduction in responses to noxious stimulus during both early and late phases of the ethanol stem bark extract of *O. barteri* at all the doses tested in formalin induced pain study as well as the acetic acid induced writhing investigations. There was a significant difference in sizes of oedema ($p < 0.05$ and $p<0.01$) between the treated right and the untreated left ears in all extracts tested. The extract exerted significant ($p<0.05$ and $p<0.01$) anti-inflammatory activity at the doses tested against egg albumin induced oedema. All the rats were healthy and active during and after the period of study. Phytochemical evaluation of *O. barteri* stem bark extract showed the presence of the following secondary metabolites; alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, while anthraquinone and phenol were not detected. The oral acute toxicity result was greater than 3089 mg/kg in mice. The findings show that ethanol stem bark extract demonstrated analgesic and anti-inflammatory activities in a dose-dependent fashion. Thus, supporting claims of the plant's traditional therapeutic importance for the treatment of pains by local population, and can be developed as an alternative therapy against the disease conditions.

Keywords: *Odina barteri*; Herbal medicine; Anti-nociceptive; Anti-inflammatory; Rodents

1. Introduction

`Pain is a distressing feeling often caused by intense or damaging stimuli. The International Association for the Study of pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with actual or potential tissue damage" [1]. The signal serves as a form of protection for organisms against potential tissue damage [2]

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Inflammation, meanwhile, is the body's immune response to injury or infection but can become problematic when chronic. Inflammation, particularly when chronic, is associated with a wide range of health issues, including autoimmune disorders, cardiovascular disease, and diabetes [3].Chronic pain, a common result of prolonged inflammation, affects approximately 1.5 billion people worldwide [4]. Inflammation-driven pain can significantly diminish quality of life, causing physical and emotional distress. In some cases, the inflammatory response, which is intended to protect the body, becomes dysregulated and contributes to disease progression.

Medicinal plants have been a cornerstone of traditional medicine for centuries, offering a rich source of bioactive compounds with therapeutic potential [5]. These plants are recognized not only for their accessibility and affordability but also for their relatively less side effects compared to synthetic agents [5]. Among the plants used in traditional medicine, *Odina barteri* has emerged as a promising candidate for addressing gastrointestinal disorders

Odina barteri, commonly known in some regions as Lannea barteri, belongs to the family Anacardiaceae. It is a deciduous tree widely distributed across West African, where it holds significant ethnomedicinal value. Traditional healers have used various parts of the plant, including the stem bark, root and leaves, to treat conditions such as ulcers, diarrhea, wounds and inflammatory diseases [6].

Despite its traditional use and promising bioactivity, there is limited scientific evidence to validate the analgesic and anti-inflammatory potential of *O. barteri* stem bark.

2. Materials and Methods

2.1. Plant Collection and Authentication

The stem barks of *Odina barteri* were sourced from a farm land in Chaze, Niger State, by an ethnobotanist, in the department of medicinal plants and traditional medicine (NIPRD, Abuja). The plant was identified and authenticated in the same department by a taxonomist.

2.2. Extraction Procedure

The stem barks of *Odinal barteri* were cleaned, cut into pieces and dried under room temperature. The dried barks were ground into powder with the aid of pestle and mortar and sieved to obtain fine powder. Thereafter, the powder was soaked in 1. 5 L of 96% ethanol and shaken vigorously for 48 hours. The mixture was filtered through Whatmann (No. 26) filter paper into a conical flask. The filtrate was later concentrated using a rotary evaporator.

2.3. Experimental Animals

Seventy-three (73) Swiss mice and Sixty (60) Adult Wistar rats, both male and female weighing between 22 -25 g (mice) and 180 to 220 g (rats) were used for the study. The animals were sourced from Animal House of Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus. The animals were kept in plastic cages (the male separated from the female) and allowed to acclimatize in laboratory environment for 14 days before commencement of study. They were allowed access to rat chow (Guinea Feeds, Nigerian PLC) and provided with clean water *ad libitum*.

2.4. Preliminary phytochemical screening

The presence of the following phytochemicals were qualitatively determined from the ethanol stem bark extract of *O.barteri,* such as terpenoids, saponins, tannins, flavonoids, cardiac glycosides, steroids and alkaloids using the method described by [7, 8].

2.5. Acute toxicity tests

The LD_{50} of the stem bark extract of *O. barteri* was tested to determine the safety of the agent following Lork's [9] method. The studies were done in two phases in Swiss albino mice. Nine mice, randomized and divided into three groups with three in each cage were used in the first phase. The mice were orally administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg of the stem bark extract respectively. In the process, animals were observed for the first 4 hours and later 24 hours for signs of toxicity and mortality which include; paw licking, weakness, feeling sleepy, respiratory distress, hyperactivity, coma and death for the first 4 hours, and subsequently 24 hours. When no signs of toxicity were observed, the second phase was arranged. In this phase, 4 mice were then grouped into 4 with one mouse per cage. Doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the stem bark extract and 10 ml/kg distilled water were administered respectively. The animals were observed for signs of toxicity and mortality for 48 hours and thereafter 72 hours for late toxicity

2.6. Analgesic activity test

2.6.1. Acetic Acid-induced Writhes

This test was done using the method described by Akuodor *et al.,* [10]. Thirty (30) Wistar albino mice of both sexes, weighing between 17-20 g were used for the study. The animals were divided into five groups of six animals each and pre-treated as follows: Group 1, which served as control, received 10 mL/kg of clean water. Group 2, 3 and 4 received 75 mg/kg, 150 mg/kg and 300 mg/kg of *O.barteri* stem bark extract, while group 5 received acetylsalicylic acid (ASA) 150 mg/kg. All treatments were administered orally (p.o). Thirty (30) minutes after pre-treatment, each mouce was given 0.7% of an aqueous solution of acetic acid (10 mL/kg). The mice were then placed in transparent Perspex observation boxes and the number of abdominal constrictions were counted for 30 minutes after treatment. The percentage inhibitions of constrictions for the extract and ASA groups were calculated as: % inhibition = (control mean – test mean/control mean X 100).

2.6.2. Formalin-induced paw licking in Rats

This was carried out as described by modified method of Akuodor *et al*., [11]. Thirty (30) Wistar albino rats of both sexes were randomly divided into 5 groups of 6 rats each. The animals were fasted for 24 hours but had access to water *ad libitum.* They were pre-treated as follows: Group 1 received clean (10 mL/kg) and this served as control. Groups 2, 3 and 4 received 75 mg/kg, 150 mg/kg and 300 mg/kg of the extract, respectively, while group 5 received 150 mg/kg ASA. All drugs were given orally. Thirty (30) minutes after treatment, all the groups were administered with 50µL of a 2.5% formalin solution, subcutaneously (S.C.) under the sub planter surface of the left hind paw. They were then placed in an observation chamber and monitored for 60 minutes. The severity of nociceptive responses was recorded based on the observation scale below:

- 0 = rats can bear weight on injected paw with free movement
- 1 = light resting of the injected paw on the floor
- 2 = partial elevation of the injected paw
- 3 = total elevation of the injected paw, licking and/or biting of the injected paw
- 4 = these observation were recorded every minute for the first 10 minutes (early phase) and then every 5 minutes thereafter up to 60 minutes (late phase).

2.7. Anti-inflammatory activity test

2.7.1. Egg albumin- induced Paw Edema in rats

Anti – inflammatory activity was assessed by the method described by Akuodor et al., [12]; Essien *et al.*, (2013) with a slight modification. Thirty (30) Swiss albino rats weighing between 180-220 g, fasted for 24 hours but allowed free access to water were used and divided in 5 groups of 6 in each group. Group1 received 10 mL/kg of clean water (free drug), group 2, 3 and 4 received the extract (75 mg/kg, 150 mg/ kg and 300 mg/kg p.o) respectively. Group 5 received the standard drug, Acetylsalicylic acid (ASA, 150 mg). After 1 hour pre-treatment with the extract, 0.2 mL of fresh egg albumin was injected into the sub-planter region of left hind paw to induce edema. The paw edema was measured initially at 0, 1, 2, 3, 4 and 5h after egg albumen injection. The difference between the initial and subsequent values gave the actual edema which was compared with control. The inhibition of inflammation was calculated using the following formula: % inhibition = 100 (VC-VT/VC) where VC represents mean edema in control and VT represents edema in group treated with drug and extract.

2.7.2. Xylene- Induced Ear Oedema in mice

Thirty (30) Swiss albino mice of both male and female selected into 5 groups of 6 mice in each cage were used in this study according to the method of Okorie et al., [14]. The animals were fasted for 24hours prior to the experiment but allowed access to water *ad libitum.* Group1 served as control and was administered with distilled water (20 mL/ kg). *O.barteri* stem bark extract (75 mg/kg, 150 mg/kg and 300 mg /kg, p. o) was given to groups 2, 3 and 4 respectively, while the standard drug, dexamethasone (4 mg/kg), was administered to group 5. One hour post drug administration, oedema was induced in each mouse by applying a drop of xylene at the inner right ear. Three hours afterwards, the animals were sacrificed under halothane anaesthesia and both ears were cut off to equal size and weighed. Inflammation was taken as mean difference between the right and left ear for each group.

3. Results

3.1. Phytochemical screening

Phytochemical evaluation of *Odina barteri* stem bark extract showed the presence of the following secondary metabolites; alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, while anthraquinone and phenol were not detected.

3.2. Acute toxicity tests

There were no lethality or toxic reactions observed at any of the doses administered. All the rats were healthy and active during and after the period of study. Hence, oral acute toxicity result was greater than 3089 mg/kg in mice and rats.

3.3. Effect of ethanol stem bark extract of *O. barteri* on acetic acid induced writhing in mice

The number of mice that writhed in response to acetic acid was significantly and dose-dependently reduced by *O. barteri* stem bark extract at $p < 0.05$ and $p < 0.01$, respectively. Compared to 75 mg/kg and 150 mg/kg, the extract's effects were greater at 300 mg/kg. This effect was similar to the prescription drug's (Table 1).

3.4. Effect of the ethanol stem bark extract of *O. barteri* on formalin test in rats

The formalin induced pain method in rats showed dose dependent analgesic effects. There was a significant ($p<0.05$ and $p<0.01$) reduction in responses to noxious stimulus during both early and late phases of the ethanol stem bark extract of *O. barteri* at all the doses tested as shown in Table 2. Aspirin, similarly showed significant ($p<0.01$) inhibition response equally in both phases compared to control.

3.5. Effect of the ethanol stem bark extract of *O. barteri* on Xylene-induced inflammation test in mice

All the ears of treated mice exhibited topical oedema induced by xylene. There was a significant difference in sizes of oedema ($p < 0.05$ and $p<0.01$) between the treated right and the untreated left ears in all extracts tested. On the effect of the extract on acute oedema, ethanol extract of *O. barteri* exhibited more inhibitory activity (Table 3). The anti-inflammatory activity is comparable to dexametasone, the standard drug which was also significant ($p<0.01$).

3.6. Effect of the ethanol stem bark extract of *O. barteri* on egg albumen-induced inflammation test in rats

The results of the effect ethanol leaf extract of *O. barteri* egg albume induced oedema in rats is shown in table 4. The extract exerted significant ($p<0.05$ and $p<0.01$) anti-inflammatory activity at the doses tested against egg albumin induced oedema. In the extract, activity was dose dependent. The reference drug, aspirin also demonstrated significant ($p<0.01$) anti-inflammatory activity.

Table 1 Effect of Ethanol stem bark extract of *Odina barteri* on acetic acid-induced Writhing Test

Treatment	Dose (mg kg)	Mean number of writhes	% Inhibition
Distilled water	10 mL kg	80.20 ± 9.18	-
Odina barteri	75	40.10 ± 4.34	50[a]
Odina barteri	150	29.40 ± 3.29	63[a]
Odina barteri	300	23.50 ± 2.25	71[b]
Aspirin	150	10.79 ± 3.43	87[b]

Results represent the mean ± SEM (n=6) a $p < 0.05$; bp < 0.01 compared to the control group

One-way ANOVA + Dunnett's post hoc test

Table 2 Effect of ethanol stem bark extract of *Odina barteri* on formalin-induced pain in rats

Treatment	Dose (mg/kg)	Early phase score of pain (0-15 min)	% Inhibition	Late phase score of pain (25-60 min)	% Inhibition
Distilled water	10 mL/kg	2.9±0.08	-	2.83±0.08	-
Odina barteri	75	1.44±0.12	49a	1.34±0.13	54a
Odina barteri	150	1.23±0.11	57a	1.16±0.06	60a
Odina barteri	300	1.04±0.51	64a	0.90±0.10	70b
Aspirin	150	0.98±0.06	65a	0.88±0.05	72b

Results represent the mean ± SEM (n=6) [a] $p < 0.05$; [b] $p < 0.01$ compared to the control group

One-way ANOVA + Dunnett's post hoc test

Table 3 The effect of ethanol leaf extract of *Odina barteri* on xylene-induced ear oedema in mice

Treatment	Dose (mg/kg)	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight (g)	% Inhibition
Distilled water	10 mL/kg	0.073±0.01	0.031±0.00	0.042±0.00	-
Odina barteri	75	0.050±0.00	0.029±0.00	0.021±0.00	50[a]
Odina barteri	150	0.038±0.00	0.025±0.00	0.013±0.00	70[b]
Odina barteri	300	0.032±0.01	0.021±0.00	0.010±0.00	78[b]
Dexamethasone	4	0.029±0.00	0.020±0.00	0.009±0.00	80[b]

Results represent the mean ± SEM (n=6) [a] $p < 0.05$; [b] $p < 0.01$ compared to the control group

One-way ANOVA + Dunnett's post hoc test

Table 4 Effect of *Odina barteri* ethanol stem bark extract against egg albumen-induced paw oedema in rats Time (hrs)

Treatment	Dose(mg/kg)	0	0.5	1	2	3	4	5
Distilled water	10 mg/kg	1.28±0.03	1.64±0.03	1.82±0.03	1.71±0.03	1.88±0.03	1.96±0.02	2.40±0.03
Odina barteri	75	1.22±0.03	1.64±0.02	1.55±0.02	1.42±0.02[a]	1.38±0.03	1.34±0.02[a]	1.26±0.02[a]
Odina barteri	150	1.20±0.02	1.61±0.02	1.53±0.02	1.47±0.02[a]	1.41±0.03[a]	1.36±0.02[a]	1.22±0.02[b]
Odina barteri	300	1.83±0.03	1.58±0.03	1.48±0.03	1.44±0.03[a]	1.32±0.04[a]	1.28±0.03[a]	1.19±0.03[b]
Aspirin	150	1.13±0.02	1.56±0.03	1.49±0.02	1.39±0.02[a]	1.30±0.02[a]	1.26±0.02[a]	1.10±0.02[b]

Results represent the mean ± SEM (n=6) [a] $p < 0.05$; [b] $p < 0.01$ compared to the control group

One-way ANOVA + Dunnett's post hoc test

4. Discussion

Chemical composition of the stem bark extract of *Odina barteri* has saponins, alkaloids, tannins, cardiac glycosides as well as flavonoids as their constituents. These results suggest that the pharmacological activities of the ethanol extract are a function of their phytochemical constituents [15]. These active principles are bioactive compound and have been reported to possess analgesic, antipyretic and anti-inflammatory properties [16].

Pain sensation is said to be one of the reasons why people ask for medical help, and therefore drugs for pains are commonly approved to relieve pain [17]. The use of analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs),

aspirin is in tandem with opionates as pain killers/relievers have not been successful in all cases as result of adverse effects such as liver damage [18]. The strategy should therefore be the search for clinically new and useful analgesics which have negligible or no side effects. The findings of the present study reveal that *O. barteri* ethanol stem bark extract at doses employed exhibited analgesic effect against chemical pains (writhings) induced by acetic acid [19]. This chemical is popular for evaluating analgesic properties of plant extracts and drugs [20]. Another report revealed that the response of mice to acetic acid is fast and trusted way to test peripheral method of analgesic effect of herbal drugs (Dutta et al., 2020). Results shows the extracts have peripheral analgesic properties which suggest the shown action probably might be directed via opposition of receptors in local peritoneal [21]. However, irrespective of whether the model evaluates peripheral analgesic action only or non-specific, the results validates the usefulness of this plant as analgesics in Nigeria. The injection of acetic acid is reported to induce prostaglandins and other cyclokinase mediators of pain [22]. This suggests that the ethanol extract of *O. barteri* acted by antagonizing cycloxygenase actions said to facilitate prostaglandins production through arachidonic acids [22]. The analgesic effects produced by the extracts of the experimental plant (*O. barteri* stem bark) validate their use in traditional herbal medicine. This extract also significantly exhibited analgesic activity. It inhibited acetic acid induced writhing response in the experimental animals. This pain mechanism is believed to involve local peritoneal recptors caused by peritoneal fluid concentration of PG-E_2 and PG-$F_{2\alpha}$ [23]. This method is not just simple and reliable but it also affords rapid evaluation of peripheral type of analgesic action. Here, the animals react in a characteristic stretching manner which is referred to as writhing. Acetic acid induced writhes is a sensitive procedure in detecting analgesic effect of medicinal agents [11]. This procedure evaluates the peripheral and central analgesic effects of the extract. Acetic acid method is simple, reliable and affords rapid examination of peripheral type of analgesic action [12]. *Odina. barteri* stem bark extract was found to inhibit acetic acid-induced writhing response in the experimental animals. The abdominal constriction is related to the sensitization of pain receptors in prostaglandins. It is therefore possible that the analgesic effect of the extract may be due to the inhibition of prostaglandins synthesis.

The formalin test has two phases; the early phase is the neurogenic phase as a result of direct stimulation of sensory nerve fibers while the late phase referred to as the inflammatory phase is caused by the release of chemical mediators including histamine, bradykinin, serotonin, and substance P [24]. This study showed that both phases were significantly reduced by the extract as compared with the control. Conventionally, centrally acting drugs are well known to inhibit both early and late phases significantly [24]. Therefore, the inhibition of both the early and late phases in this test together with the reduction of abdominal constrictions in the acetic acid-induced writhing test showed that the extract of *Odina barteri* stem bark acts centrally and peripherally [25].

Due to the need of additional tests to overcome the low specificity of the acetic acid-induced abdominal writhing test, and also aiming to better characterize the antinociceptive activity of the *O. barteri*, the formalin-induced paw licking test in rats, a chemical model of nociception which provides a more specific response [26] and is considered the closest model for clinical pain [27]. The application of the irritant compound into the hind paw makes the nociceptive response more specific, since during grooming the animals most frequently use their forelegs [28]. The formalin-induced paw licking test demonstrates two distinct phases of nociceptive behavior which seem to involve different mediators (Piccinelli et al., 2015). The first phase of nociception starts immediately after formalin injection, extending for the following 5 min, and is believed to occur due to direct chemical stimulation of nociceptors [29]. This first phase is inhibited by opioid agonists, such as morphine and fentanyl, by bradykinin B1 and B2 receptor antagonists, by N-methyl-D-aspartic-acid (NMDA) receptors, as well as by vanilloid receptor antagonists [26]. The second phase of this model takes place 15 to 30 min after formalin injection and is related to the release of several proinflammatory mediators [30]. It was observed that both doses especially the highest dose (300 mg/kg) strongly inhibited both the first and the second phase of the formalin model, and therefore confirmed that *O. barteri* is endowed with potent antinociceptive and/or anti-inflammatory activity.

The suitable way of testing anti-inflammatory action of natural products is by using [31] method. This process of testing acute inflammation potency is a highly sensitive tool [31]. *O. barteri* stem bark extract significantly inhibited development of egg albumin paw induced edema in three phases of the test. However, the inhibitory effects were more prominent in the second and third phases. Oedema development may result from the operation of different mediators in order to bring about inflammatory response [32]. According to these authors, bradykinin is one of the early mediators while prostaglandins are seen in final phase of inflammation. Nonsteroidal anti-inflammatory agents like aspirin, may not inhibit the initial phase of edema induced by egg albumen whereas the second accelerating phase can be antagonized by the drug [32]. Infiltrations of local neutrophil contribute to inflammatory response giving rise to mediators like superoxide anion (O^{2-}) and hydroxyl radicals. Induced reduction of COX-2 is associated with diminution swelling witnessed in induced paw from egg albumen [31]. *Odina barteri* stem bark extract effect in egg albumin-induced rat paw edema test in this study suggest that it elicits anti-inflammatory activity possibly via inhibition of production of prostaglandins and diminution of inducible cyclooxygenase, NO and other mentioned cytokines. However, specific

mechanism(s) of action tests needs to be carried out to establish this possible effect. This study indicated that ethanol extract of *O. barteri* inhibited egg albumin-induced suplanter oedema in mice. Egg albumin-induced mice paw oedema is a valuable test used in predicting the value of anti-inflammatory agents acting by inhibing the mediators of acute inflammation. A lot of substances have been proposed as inflammatory mediators, released locally at the site of inflammation and having biological properties that cause or enhance the signs and symptoms of inflammation.

Perturbation of the neutrophil membrane is an important event elicited by an inflammatory stimulus. This usually produces highly reactive oxygen species such as superoxide. The effect of extract seems to coincide with the nonphagocytic phase of egg albumin – induced inflammation, when the mast cells release cytoplasmic enzymes and serotonin [33].

Xyelene is known to cause irritation in mouse ear which leads to accumulation of fluid and oedema, characteristic of acute inflammatory response. Suppression of this response may indicate antiphlogistic effect [12]. The stem bark extract of *Odina barteri* exhibited a significant inhibition of ear oedema caused by xylene. This activity suggests the inhibition of phospholipase A_2 which is involved in the pathophysiology of inflammation due to xylene [34]. The anti-inflammatory activity of dexamethasone may be due to the presence of flavonoids, saponins, tannins, triterpenes and alkaloids. The inhibition was dose-dependent. The inhibition may have be initiated by the presence of one or some of the bioactive compounds contained in the extract. In rheumatoid arthritis, flavonoids were reported to inhibit the release of chemical mediators through the histamine while serotonin reduces the symptoms. These were thought to be mediated through decreased monocyte infiltration and fibroblast proliferation, blocked TNF-X and inhibition of COX. The mode of action of the anti-inflammatory potential of the plant extract may have probably followed the same pattern.

The mechanism of anti-inflammatory activity for the revealed phytoconstituents in *O. barteri* has been reported in literature. One of such is through the inhibition of NF – KB activation and down-regulation of the expression of inflammatory enezyme markers such as 5-COX, COX-2 and MMP-9 [34]. The results of the phytochemical screening of *O. barteri* showed that it contains biological active substances including tannins and flavonoids with potential values for the treatment of inflammation and painful conditions.

5. Conclusion

The current study's findings support the plant's traditional applications for treating various illnesses by demonstrating the analgesic, anti-inflammatory, and antipyretic properties of the ethanol stem bark extract of *Odina barteri*

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest associated with authors of this article.

Statement of ethical approval

The study was conducted according to the Animal Ethical Committee Guidelines of Nnamdi Azikiwe University, Awka, Anambra State (NAU/AREC/2025/0191), as well the National Institute of Health Guide for the care and use of Laboratory Animals.

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