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Protective effects of methanolic extract of *Bauhinia vahlii* L. in sepsis rats induced by cecal ligation and puncture

Tác dụng bảo vệ của chiết xuất methanol của *Bauhinia vahlii* L. ở chuột bị nhiễm trùng huyết do thắt và thủng manh tràng

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Abstract

The present study investigated the phytochemical, antibacterial, acute toxicity properties, hemodynamic parameters, and myeloperoxidase activity of methanolic extract of *Bauhinia vahlii* **(Bv)**, and its effects on cecal ligation and puncture (CLP)-induced sepsis in mice. The preliminary phytochemical screening showed that **Bv** contains alkaloids, terpenoids, flavonoids, phenolics, saponins, and tannins. At equivalent concentration, **Bv** showed antibacterial activity as potent as streptomycin against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. Acute toxicity studies on mice found out that **Bv** was non-toxic up to 2000 mg/Kg body weight. At both low and high doses, **Bv** improved hemodynamic parameters including mean arterial pressure, optical density of blood, and serum myeloperoxidase activity. Moreover, **Bv** improved the survival rate of sepsis mice (83.34% at the low dose and 66.67% at the high dose) as compared to the untreated ones (16.67%), possibly due to its anti-inflammatory effects. The results indicated that *B. vahlii* can be used as a favorable natural source for the treatment of CLP-induced sepsis.

Keywords: Anti-bacterial activity; CLP-induced sepsis; hemodynamic parameters; myeloperoxidase activity.

Tóm tắt

Nghiên cứu này kiểm tra các đặc tính hoá thực vật, kháng khuẩn, độc tính cấp tính, các thông số huyết động, và hoạt tính myeloperoxidase của chiết xuất methanol của *Bauhinia vahlii* (**Bv**), và tác động của nó trên nhiễm trùng huyết do thắt và thủng manh tràng (CLP) ở chuột. Sàng lọc hóa thực vật sơ bộ cho thấy **Bv** có chứa alkaloid, terpenoit, flavonoit, phenolic, saponin và tannin. Ở nồng độ tương đương, **Bv** thể hiện hoạt tính kháng khuẩn mạnh tương đương streptomycin trên các chủng *Staphylococcus aureus*, *Salmonella typhi*, và *Escherichia coli*. Các nghiên cứu về độc tính cấp tính trên chuột cho thấy **Bv** không gây độc ở nồng độ lên đến 2000 mg/Kg thể trọng. Ở cả liều thấp và liều cao, **Bv** cải thiện các thông số huyết động học như áp lực động mạch trung bình, độ mật độ quang của máu, và hoạt tính myeloperoxidase huyết thanh. Hơn nữa, **Bv** giúp cải thiện tỷ lệ sống sót ở chuột bị nhiễm trùng máu (83.34% ở liều thấp và 66,67% ở liều cao) so với

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nhóm không được chữa trị (16.67%), có thể là nhờ hoạt tính kháng viêm của nó. Các kết quả này cho thấy *B. vahlii* có thể được sử dụng như một loại thuốc từ thiên nhiên để điều trị nhiễm trùng huyết do CLP.

Từ khoá: Hoạt tính kháng khuẩn; nhiễm trùng huyết do thắt và thủng manh tràng; các thông số huyết động học; hoạt tính myeloperoxidase.

1. Introduction

Sepsis is a lethal clinical condition that is one of the major causes of death in intensive care units worldwide [1]. This condition is caused by the dysregulated systemic inflammatory response of the body due to the invasion of pathogens [2]. The complications of sepsis are largely varied and generally involved in coagulation disorders, immune suppression, organ dysfunction, and systemic inflammation [3,4]. Severe sepsis might affect the cardiovascular system such as cardiomyopathy and endothelial dysfunction as a result of the adverse effects of substances secreted from pathogens and host cells [5].

Sepsis impairs neutrophil migration and its antimicrobial activity. Inadequate migration of neutrophils into the site of infection causes the systemic spread of pathogens, which results in high rates of mortality. The initial management of infection in the sepsis requires a timely and appropriate antibiotic therapy [6]. However, to date, there is still no specific drug/therapy against sepsis. Hence, searching for a new medication from herbs and medicinal plants for the treatment of sepsis is necessary.

Bauhinia genus belongs to family Fabaceae, well recorded in the flora of India, Nepal, and Pakistan [7]. *Bauhinia vahlii* is a strong climbing shrub that is usually called “Camel’s foot creeper” in English [8]. In the folklore, *Bauhinia* species has wide applications in the treatment of microbial infections, oxidative stress, inflammation, diabetes and tumors. Particularly, this plant was commony used in the treatment of microbial infections, oxidative stress, chronic inflammation, and cancer in the Indian tribes. Biologically, *B. vahlii* reported for antibacterial [8,9], antioxidant [10,11], anti-inflammatory [12], tyrosinase inhibitory [11] and anti-diabetic [12] activities. Besides, a chemical examination on leaves of *B. vahlii* reported the presence of triterpenes, flavonoids, phenolic acids, and sterols [13]. Therefore, in the current study, we aimed to evaluate the phytochemical analysis, and antibacterial activity of the whole plant *B. vahlii* extract, as well as its protective effects on hemodynamic parameters, myeloperoxidase (MPO) activity and survival rate in cecal ligation and puncture (CLP)-induced sepsis in mice.

2. Material and methods

2.1. Collection

The whole plant of *Bauhinia vahlii* L. was collected at Seshachalam hills, Tirupati, Andhra Pradesh, India, in 2019, and a voucher specimen (DB-SVU-2019-3478) has deposited at Department of Botany, Sri Venkateswara University, India.

2.2. Extraction

The whole plant was dried and powdered (200 g) and extracted three times with methanol 96% at 25˚C. All combined and evaporated under low pressure to obtain a methanolic extract of *B. vahlii* (**Bv,** 2.0 g), which was preserved in an amber color bottle at 4 ˚C [14].

2.3. Preliminary phytochemical analysis

Preliminary phytochemical analysis upon **Bv** was performed according to the standard practical methods [15,16].

2.4. Antibacterial activity

In vitro antimicrobial activity of **Bv** was performed by the cup-plate method [17]. The obtained extract was tested against two gram-positive bacteria (*Staphylococcus aureus* (ATCC25923) and *Bacillus subtilis* (ATCC21332)) and two gram-negative bacteria (*Salmonella typhi* (ATCC1408) and *Escherichia coli* (ATCC25922)). Mueller Hinton agar plates inoculated with 0.5 McFarland standards of mentioned bacteria were used for this assessment. Tested strains were inoculated by spread plate technique, and wells were made by sterile cork borer. After that, 50 µl of **Bv** and the standard streptomycin (100 µg/ml) were applied to each well. After 24 h incubation at 37°C, inhibition zones were measured by calibrated scale [18].

2.5. Animals

Adult male mice (weighting 25±5 mg, age 6-8 weeks) were used in this study. The animals were given food and water *ad libitum* and were housed in the standard condition with a temperature of 21±2 °C, the relative humidity of 50±10% and a 12-h light/12-h dark cycle [19]. This study was approved by the Ethics Committee of Andhra University College of Pharmaceutical Sciences (Code: AUCOPS.2020.442).

2.6. Acute oral toxicity

Mice were randomly divided into 4 groups (6 mice in each group). The OECD main test 425 (up-and-down dose procedure) was utilized using doses of 175, 550, 1750, and 2000 mg/kg body weight (b.w) of **Bv**. The tested animals have undergone fasting overnight before administering the extract using oral gavage. The first set of tested animals was administered with a dose of 175 mg/kg b.w. When the tested animal survived after 48 h, the dose that was given to the next sets of rodents was increased by a factor 3.2, meaning that a dose of 550 mg/kg b.w, 1750 mg/kg b.w, and the upper bound dose of 2000 mg/kg b.w was given to the tested rodents. The test was ended when only the last three animals survived with the upper bound dose, and all of the test animals were observed up to 14 days [20,21].

2.7. Cecal ligation and puncture (CLP)-induced sepsis in mice

CLP-induced model [22] was used for the induction of sepsis. At the beginning of the experiment, mice were randomly divided into 4 groups (6 mice in each group). Mice in group 1 (normal control) underwent midline abdominal incision without CLP. Mice in group 2 (CLP-induced) underwent midline abdominal incision with cecal ligation (50%) and punctured to induce polymicrobial sepsis. Mice in groups 3 and 4 received 100 mg/kg b.w (as a low dose) and 200 mg/kg b.w (as a high dose) of **Bv** intraperitoneal (i.p) at 0, 1, 3, 6 and 24 h after CLP-induced operation. Blood samples were obtained from the portal vein. 0.5 ml of blood samples were transferred into laboratory tubes containing pre-autoclaved nutrient broth medium (Sigma-Aldrich, Germany) and incubated at 37 °C. The remaining blood samples decanted gently into collection plastic tubes, then centrifuged at 3000 rpm for 5 min. Then serum was obtained, aliquoted into micro tubes, and stored at -20 °C for biochemical analysis.

Later, mice were anesthetized by i.p injection of ketamine (60 mg/kg b.w) and xylazine (10 mg/kg b.w). Then, the abdominal region of animals was shaved and sterilized by betadine. The cecum was exposed through a midline abdominal incision and ligated (50 %) with 3/0 silk suture then punctured with a sterile 18-gauge needle. The cecum was gently squeezed and after a drop of cecal contents was discharged, the cecum was repositioned into the abdominal

cavity. The abdominal wall and skin were closed with 3/0 silk suture. After the surgery, mice received 3 ml of warm 0.9% normal saline subcutaneously (s.c) for fluid resuscitation. After mice recovered from anesthesia, they had free access to food and water.

2.8. Animal survival rate

In addition to monitoring the animals for three days, animals' survival rate was reported after 72 h [23].

2.9. Hemodynamic parameters

For measurements of hemodynamic parameters such as arterial blood pressure, mean arterial blood pressure, developed pressure and heart rate, a polyethylene cannula connected to a pressure transducer that prefilled with heparinized normal saline solution was cannulated into the right common carotid artery [23].

2.10. Myeloperoxidase (MPO) measurement

The activity of MPO [24], an abundant enzyme of neutrophils, was assessed as previously described with minor modification. Briefly, 1 ml of the serum was mixed with 1 mg of hexadecyltrimethylammonium bromide (HTAB) followed by sonication for 5 min and centrifuged at 3000 rpm for 10 min at 4 ˚C. Then, a mixture of 0.1 ml of supernatant with 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml O-Dianisidine dihydrochloride and 1% hydrogen peroxide was incubated for 5 min at room temperature. After adding 0.1 ml of 1.2 M HCl, the change in absorbance was measured at 460 nm using a spectrophotometer.

3. Results

3.1. Phytochemical analysis

Results of the preliminary phytochemical screening of **Bv** showed that this plant possesses alkaloids, terpenoids, flavonoids, phenolics, saponins, and tannins. Coumarins, phenanthrenes, anthraquinones, bibenzyls, fluorenones, and cardiac glycosides were totally absent in the extract (Table 1).

Table 1: Phytochemical analysis of methanolic extract of *B. vahlii* **(Bv)**

No	Phytochemical	Methanolic extract of *B. vahlii* (Bv)	No	Phytochemical	Methanolic extract of *B. vahlii* (Bv)
1	Alkaloids	+	7	Fluorenones	-
2	Anthraquinones	-	8	Phenanthrenes	-
3	Bibenzyls	-	9	Phenolics	+
4	Cardiac glycosides	-	10	Saponins	+
5	Coumarins	-	11	Tannins	+
6	Flavonoids	+	12	Terpenoids	+

"+" indicates presences; "-" indicates absence

3.2. Acute oral toxicity (OECD main test 425)

Bv was found to be non-toxic up to 2000 mg/kg b.w of tested mice. There were no significant changes in the pattern of behavior of the tested animals and no mortality was noted for 14 days. These results signified that the **Bv** extract is non-toxic up to 2000 mg/kg b.w, and the low (1/20) and high (1/10) dosage were fixed as 100 and 200 mg/kg b.w, respectively.

3.3. Antibacterial activity

The *in vitro* antimicrobial assays of **Bv** extract revealed that it has antibacterial activity

against both gram-positive (*S. aureus* and *B. subtilis*), and gram-negative (*S. typhi* and *E. coli*) bacteria (Table 2). In particular, **Bv** extract was found to be as potent as the standard streptomycin against *S. aureus*, *S. typhi*, and *E. coli* with equal zone of inhibitory effect. The inhibition capacity of Bv extract on *B. subtilis* was lower than that of streptomycin, although the zone of inhibition still high (23.0±0.2 mm).

Table 2: Antibacterial screening test of methanolic extract of *B. vahlii* (Bv)

Sample	Zone of inhibition (mm)*			
	Gram-positive		Gram-negative	
	B. subtilis	***S. aureus***	***E. coli***	***S. typhi***
Bv	23.0±0.2	21.5±0.1	16.0±0.1	20.0±0.1
Streptomycin	26.5±0.1	22.9±0.1	17.3±0.1	20.2±0.1

*mean±SD values (n = 3)

3.4. Optical density of blood

Our results shoed that the optical density (OD) of blood was significantly (p < 0.01) increased in the CLP-induced group as compared to the normal control group (Figure 1). On the other hand, the administration of **Bv** (at both low and high doses) to the septic mice had significantly decreased the OD of the blood of animals as compared to that of the CLP-induced group in a dose-dependent manner (Figure 1).

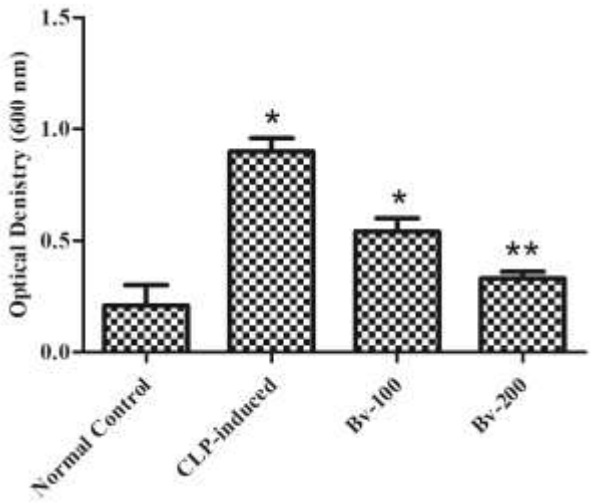

Figure 1. Effect of methanolic extract of *B. vahlii* **(Bv)** on the OD of the blood samples. Values are presented as mean±SD (n = 6); *p < 0.05, **p < 0.01. Statistical analysis was done using one-way ANOVA with Student-Newman-Keuls *post hoc test*; Bv-100: **Bv** at 100 mg/kg body weight; Bv-200: **Bv** at 200 mg/kg body weight.

3.5. Hemodynamic responses

It was observed that the mean arterial pressure was significantly decreased from 113.84±6.17 mm of Hg in the normal control group to 51.0±3.0 mm of Hg (-57.44%) in the CLP-induced group (p < 0.05). The treatment of CLP-induced mice with **Bv** at 100 and 200 mg/kg had significantly improved (p < 0.05) the mean arterial pressure to 87.67±2.34 (-26.85%) and 108.5±5.5 (-9.46%) mm of Hg, respectively. Similarly, the arterial blood pressure from 148.0±8.0 mm of Hg in the normal control group decreased to 73.34±4.67 mm of Hg (-50.45%, p < 0.001) in the CLP-induced group (Table 3). Treatment with **Bv** at 100 and 200 mg/kg b.w had also increased the arterial blood pressure of the CLP-induced mice (p < 0.001) significantly with 117.17±8.84 (-20.83%) and 120.5±5.5 (-18.58%) mm of Hg, respectively.

On the other hand, the heart rate was increased insignificantly in the CLP-induced group (+21.40%) as compared to the control group. Treatment with **Bv** at 100 and 200 mg/kg b.w help to slow down the heart beat of the CLP-induced mice and bring their heart rate close to that of the normal control group with the heart rate of 226.34±7.67 (+9.69%) and 210.50±5.5 (+2.02%), respectively (Table 3).

Developed pressure was significantly decreased in the CLP-induced group (-43.92%) as compared to the control group, and **Bv** treated at 100 and 200 mg/kg b.w was substantial reversed the effects on the CLP-induced mice (-9.58 and -16.09%, respectively) (Table 3).

Table 3: Effects of methanolic extract of *B. vahlii* **(Bv)** on hemodynamic parameters in CLP-induced sepsis after 72 h

Sample	Hemodynamic parameters (mm of Hg)*			
	Mean arterial pressure	**Arterial blood pressure**	**Heart rate**	**Developed pressure**
Normal Control	119.84±6.17	148.0±8.0	206.34±9.67	38.34±3.67
CLP	51.0±3.0[a] (-57.44%)	73.34±4.67[b] (-50.45%)	250.5±13.5 (+21.40%)	21.5±2.5[a] (-43.92%)
Bv-100	87.67±2.34[c] (-26.85%)	117.17±8.84[d] (-20.83%)	226.34±7.67 (+9.69%)	27.0±3.0 (-29.58%)
Bv-200	108.5±5.5[d] (-9.46%)	120.5±5.5[c] (-18.58%)	210.50±5.5 (+2.02%)	32.17±3.84 (-16.09%)

*mean±SD values (n = 6); one-way ANOVA with Student-Newman-Keuls *post hoc test* was used for wise pair comparison where [a]$p < 0.05$, [b]$p < 0.001$ as compared to the normal control group; [c]$p < 0.05$, [d]$p < 0.001$ compared with CLP group. Bv-100: **Bv** at 100 mg/kg body weight; Bv-200: **Bv** at 200 mg/kg body weight.

3.6. Serum MPO activity

Animals in the CLP-induced group showed a significant increase in MPO activity as compared with the normal control group ($p < 0.05$). The treatment of CLP-induced mice with **Bv** (at both low and high doses) had decreased markedly ($p < 0.01$) the enzyme activity compared with the untreated CLP-induced group (Figure 2).

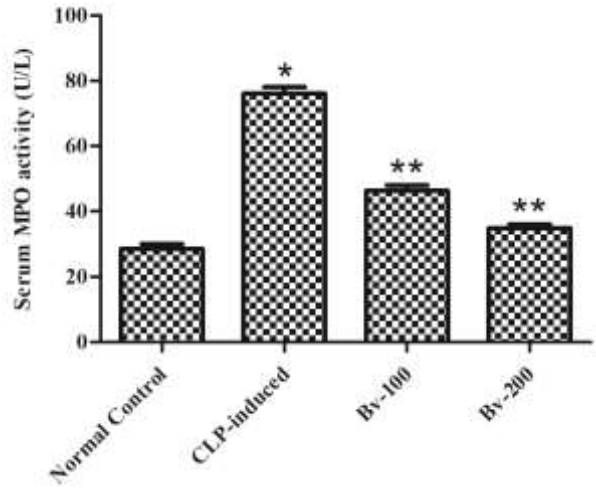

Figure 2. Effect of methanolic extract of *B. vahlii* **(Bv)** on MPO activity. Values are presented as mean±SD (n=6); *$p < 0.05$, **$p < 0.01$. The statistical analyses were done using one-way ANOVA with Student-Newman-Keuls *post hoc test*. Bv-100: **Bv** at 100 mg/kg body weight; Bv-200: **Bv** at 200 mg/kg body weight.

3.7. Survival rate

To examine the effects of **Bv** on the survival rates, the animals were monitored for 72 h after CLP-induced surgery. There was no death of mice in the normal control group after 72 h, meaning the survival rate of mice in this group

was 100.0%. On the other hand, at 72 h, the survival rate in the CLP-induced group was decreased to 16.67% as compared to the normal control group. Treatment of septic mice with **Bv** with doses of 100 and 200 mg/kg b.w improved their survival rate at 72 h to 83.34% and 66.67%, respectively (Figure 3).

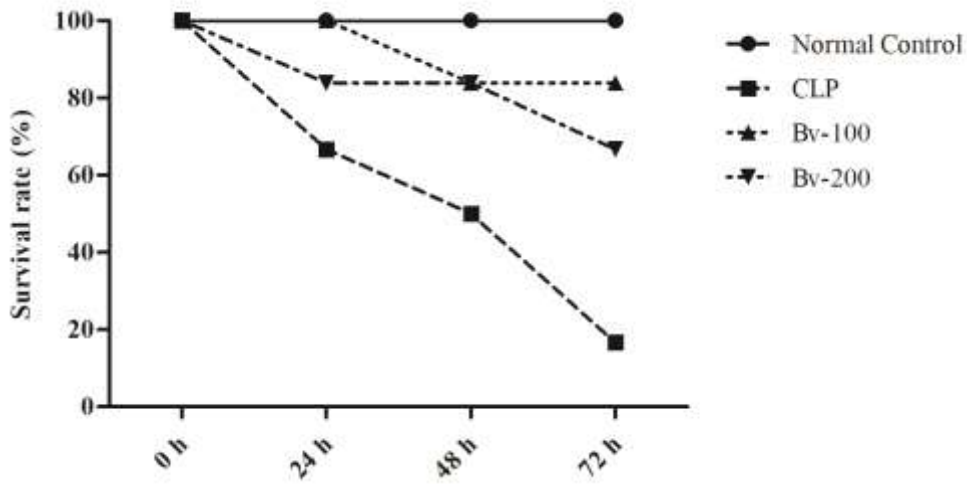

Figure 3. Effect of methanolic extract of *B. vahlii* **(Bv)** on survival rate after 72 h (n = 6). Values are presented as mean±SD (n=6); Bv-100: **Bv** at 100 mg/kg body weight; Bv-200: **Bv** at 200 mg/kg body weight.

4. Discussion

In the present study, we investigated the phytochemical properties and bioactivities of **Bv** extreact. Our ressults showed that **Bv** contains alkaloids, terpenoids, flavonoids, phenolics, and tannins, with a potent antibacterial activity and ability to reduce the inflammatory response and mortality rate in mice with polymicrobial sepsis.

Bv was found to has antibacterial activity against gram-positive (*S. aureus* and *B. subtilis*), and gram-negative (*S. typhi* and *E. coli*) bacteria. As *S. aureus* and *E. coli* bacteria are the predominant sepsis-caused pathogens, this edible flower might have beneficial effects against sepsis [25]. The antibacterial activity of **Bv** might be related to its phytochemical contents since the antibacterial activities of alkaloids, flavonoids, and phenolic acids, the main components of **Bv**, have been previously reported [25,26].

It has been noted with evidence that sepsis may causes cardiac and endothelial dysfunction [5,26]. The hemodynamic monitoring in this study showed the attenuation of hemodynamic parameters in the CLP-induced group. This event can leads to a misbalance in tissues oxygen supply/demand and accelerates the process of septic shock. For this reason, apart from antibiotic therapy, hemodynamic stability is essential in the management of sepsis [6]. Administration of **Bv** (at both low and high doses) to the septic mice had significantly (p < 0.05) decreased the OD of the blood of CLP-induced mice as compared to that of the untreated ones (Figure 1). In addition, administration of **Bv** to septic mice had increased the mean arterial and blood pressure as compared to the untreated CLP-induced group (Table 3). These results suggested that the administration of medications-extracted from *B. vahlii* might be useful for the treatment of sepsis.

MPO, the major enzyme in azurophilic granules of neutrophils, is a marker of inflammation initiation in plasma. Thus, the increased MPO activity indicates the onset of the inflammatory response and neutrophil infiltration due to the induction of microbial sepsis [24]. Our results showed that MPO activity has increased in the serum of mice with CL-induced poly-microbial sepsis in comparison with the normal ones. However, this effect was reversible upon the asdministration of **Bv** on the CLP-induced mice due to its anti-inflammatory effect (Figure 2). In addition, the present study showed a reduction in survival rate in CLP-induced sepsis in mice, mostly due to the excessive release of cytokines, which results in a hyper-inflammatory state. Treatment of sepsis mice with **Bv** have subtentially improved the survivial rate.

5. Conclusion

The results of the present study is the first report on the plant *B. vahlii* in the treatment of CLP-induced sepsis in mice. The key phytochemicals responsible for this activity claimed to be alkaloids, flavonoids, polyphenols, and tannins. The results provide the evidence that supports the traditional uses of *B. vahlii*. Moreover, these findings suggest that plant *B. vahlii* can be used as a good natural source of remedial medicine for sepsis. Further studies to identify the potential bioactive molecules from *B. vahlii* that are responsible for these effects may aid in drug discovery for this condition.

Conflict of interest

No conflict of interest between any of the authors.

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