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ANTI-ASTHMATIC ACTIVITY OF THE ROOT EXTRACTS OF *DESMODIUM GANGITECUM* DC

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Abstract

*Bronchial asthma is characterized by hyper responsiveness of trachea bronchial smooth muscle to have a variety of stimuli, resulting in narrowing of air tubes, often accompanied by increased secretion, mucosal edema and mucus plugging. Symptoms include dyspnoea, wheezing, cough and may be a limitation of activity. An allergic basis can be demonstrated in many adult and higher percentages of pediatric patients. In others, a variety of trigger factors (infections, irritants, pollution, exercise, exposure to cold air and pschycogenic) may be involved[2]. The preliminary phytochemical analysis revealed that the dried root chloroform, ethanolic and aqueous alcoholic extracts of the plant Desmodium gangitecum DC, Family-Fabaceae were screened for their preliminary phytochemical analysis, in which it showed the presence of alkaloids, carbohydrates, glycosides, phytosterol and flavonoids. The pharmacological screenings of these extracts revealed that there was a significant decrease in WBC count for extract-treated rats as compared to sensitized control II (Ovalbumin) treated rats and the significance was observed for the estimation of total tissue protein content as compared to sensitized control II treated rats. The extracts showed an significant results of tissue Malonyldialdehyd**e (***MDA) levels in the experimental rats as compared to sensitized control II treated rats and this study indicates the veracity of anti asthmatic activity claimed by the natural medical practitioners of The Nilgiris.*

Key words: Asthma, Herbal Extracts, Ovalbumin, Malondialdehyde.

Introduction

The *Mullukurumbas* community tribal traditional practitioners in Gudalur, The Nilgiris are using this herbal drug *Desmodium gangeticum DC (*family- Fabaceae) as a drug of choice for asthmatic disorder. Asthma is one of the major diseases, which affects the human kind to include people of all ages, viz- neonates to geriatric patients in the world. The traditional healers disclosed that this drug is giving promising clinical results against asthma[1].

Bronchial asthma is characterized by hyper responsiveness of trachea bronchial smooth muscle to have a variety of stimuli, resulting in narrowing of air tubes, often accompanied by increased secretion, mucosal edema and mucus plugging. Symptoms include dyspnoea, wheezing, cough and may be a limitation of activity. An allergic basis can be demonstrated in many adult and higher percentages of pediatric patients. In others, a variety of trigger factors (infections, irritants, pollution, exercise, exposure to cold air and pschycogenic) may be involved[2].

Plant profile[3,4]

The plant consists of dried roots of ***Desmodium gangitecum* DC. Family-Fabaceae**

Desmodium gangitecum DC plant

Desmodium gangeticum DC root

Figure: The plant of *Desmodium gangeticum DC*

Description[3,4]**:**

It is a small plant having a height of 2 to 4 feet, the stem is angular. Leaves are ovate in shape that is 3 to 6 inch in length. The lower surface of the leaf is of light green in color. Flowers are purple or white in color. The plant bears flowers and fruits throughout the whole year especially in early summers. The plant has many long lateral roots arising nearly from the base of the stem. This plant contains alkaloids such as indole-3-alkyamine and their oxides and 6-methoxy-2-methyl-carboline, indoles and 5-oxyindoles, vitamins, oils and minerals like calcium, phosphorus and magnesium. Ethno-pharmacologically *Desmodium gangitecum* DC plant is used as alternative, anthelmintic, anti catarrhal, carminative, diuretic, expectorant, febrifuge, nerve tonic, antidiarrheal, stomach ache and tonic. It is also used for inflammation, vomiting, brain affections and scorpion sting, and Asthma. The plant was collected from Gudalur, The Nilgiris and authenticated.

Objective

Mullukurumbas Tribal community traditional practitioners in Gudalur, The Nilgiris are using this herbal drug *Desmodium gangitecum* DC *(*family: Fabaceae*)* as a drug of choice for asthmatic disorder. It was proposed to carry out the scientific validation of the dried roots of *Desmodium gangitecum* DC for their anti asthmatic activity.

Methodology

Pharmacognostical Studies

The dried root powders of *Desmodium gangitecum* DC where extracted successively with chloroform, ethanol and hydro alcoholic solvents, extracts were dried and subjected to analyse the preliminary phytochemical analysis with various solvents and reagents.

Pharmacological Studies

i. Gross behavioral and toxicity studies of the dried root extracts of *Desmodium gangitecum* DC[16]

The Gross behavioral and toxicity studies were carried out for the dried root extracts of *Desmodium gangeticum* DC using Swiss albino mice of either sex and the results were recorded.

ii. *In vivo* anti-asthmatic activity of the dried root extracts of *Desmodium gangitecum* DC[17]

Thirty six Wistar rats weighing 180-125g were divided into six groups of six animals in each group of either sex. Animals were provided with standard diet and water *ad libitum*. The food was withdrawn 18-24 hours before the start of the experiment.

Ovalbumin induced- asthmatic rats

All the rats were weighed before treatment and grouped as follows:

Group I : received equivalent amount of normal saline 1ml/kg body weight (Served as solvent control) 30 rats (**GroupII to Group VI**) were rendered asthmatic by daily administration of Ovalbumin 5mg/kg body weightintra muscularly (i.m.) for consecutive 21 days. .

Group II : continued to receive only ovalbumin 5mg/kg body weight i.m. (Control) for 30 days,

Group III : received ketotifen 5mg/kg body weight dissolved in distilled water along with ovalbumin for 30 days i.m.

Group IV :received 200mg/kg, body weight chloroform root extract of *Desmodium gangitecum* DC, **(**DGCRE**)** dissolved in distilled water along with ovalbumin for 30 days i.m.

Group V : received 200mg/kg, body weight ethanolic root extract of *Desmodium gangitecum* DC, **(**DGERE**)** dissolved in distilled water along with ovalbumin for30 days i.m.

Group VI : received 200mg/kg, body weight aqueous alcoholic root extract of *Desmodium gangitecum* DC, **(**DGARE**)** dissolved in distilled water along with ovalbumin for 30 days i.m.

On the last day, after overnight fasting, all the animals were weighed anesthetized and the blood samples were collected for differential count (DC) and for white blood cell (WBC) count. The results are tabulated.

Then the anesthetized animals were sacrificed. Their lung samples were collected and used for estimation of protein and malondialdehyde content

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Estimation of differential cell count total and white blood cellcount

The broncho alveolar lavage fluid (BALF) was collected and centrifuged at 170 G for 10 minutes. The pellets obtained after centrifugation were resuspended in 0.5ml of phosphate buffer solution (PBS) and a thin smear was prepared on a slide. Then the smeared slide was air-dried and stained for ten minutes using Giemsa stain. The slide was washed with distilled water for the purpose of de-staining. Counter staining was later carried out by using May Grunwald stain. The differential count was carried out using a digital light microscope (Motic, Japan, Cat. No.B-1series) at 100X magnification by oil immersion technique. The results are tabulated.

Estimation of Total protein from lung tissue homogenate[18]:
Estimation of protein was done by Lowry's method, which requires four different solutions namely: solution A, solution B, solution C, and solution D.
Solution A is the mixture of sodium carbonate (2%), sodium tartarate (0.05%), and sodium hydroxide (4%).
Solution B contains freshly prepared copper sulphate solution (1%).
Solution C contains 9 parts of solution A mixed with 1 part of solution B and solution D contains the Folins-Ciocalteaus reagent.

Step I- 0.2, 0.4, 0.6, 0.8ml of the homogenate supernatant was pipetted out and 1ml of the working standard was added into a series of test tubes.
Step II- 0.1ml and 0.2ml of the sample extract was pipetted out into two other test tubes.
Step III- The volume was made up to 1 ml in all the test tubes with double distilled water and a test tube with 1ml of water served as the blank.
Step IV- 5 ml of reagent C was then added to each tube including the blank. They were then mixed well and allowed to stand for 10 minutes.
Step V- 0.5 ml of reagent D was then added, mixed well and incubated at room Temperature in dark for 30 minutes till a blue color develops.
Step VI- Readings were taken at 532 nm.
Step VII- a standard graph was plotted and the amount of protein in the sample was calculated. The results are tabulated.

Estimation of Malonyldialdehyde (MDA) from lung tissue: An incubation mixture was prepared as shown in the following table. The incubation mixture were consisting of :-

Ingredients	**Volume**
Tissue homogenate	0.5ml (lung)
8.1 sodium dodecyl sulphate (SDS)	0.2ml
20% acetic acid solution (adjusted to pH 3.5 with NaOH /0.1N HCl solution)	1.5ml
0.8% aqueous solution of thiobarbituric acid to pH 7.4 with NaOH /0.1N HCl solution)	1.5ml

The incubation mixture was made up to 5 ml with double distilled water and then heated in boiling water bath for 30 minutes. After cooling, the rat chromogen was extracted into 5ml of a mixture of n-butanol and pyridine (15:1 v/v), centrifuged at 4000 rpm for 10minutes.The organic layer was then separated and its absorbance was measured at 532nm. 1, 1, 3, 3-tetra ethoxypropane (TEP) was used as an external standard and the level of lipid peroxides was expressed in nmoles of lipid peroxidation units /mg tissue. The calibration curve for TEP was prepared by the above described procedure taking TEP as standard. Linearity was obtained over the range of 80-240 nmoles of TEP.

$$\text{MDA content} = \frac{\text{Absorbance X Extract Volume X Pathlength (1 cm)}}{1.56 \text{ X } 10^{-5} \text{ X Protein Content}}$$

The results are tabulated.

Results
Pharmacognostical Studies
The preliminary phytochemical analysis revealed that the dried root chloroform, ethanolic and aqueous alcoholic extracts of the plant *Desmodium gangeticum* DC were screened for their preliminary phytochemical analysis, in which it showed the presence of alkaloids, carbohydrates, glycosides, phytosterol and flavonoids.

***In vivo* antiasthmatic activity of the dried root extracts of *Desmodium gangeticum* DC:**
The gross behavioural toxicity study revealed that the various root extracts of *Desmodium gangeticum* DC. showed no toxic effect at the tested dose level of a maximum of 2000 mg/Kg body wt.

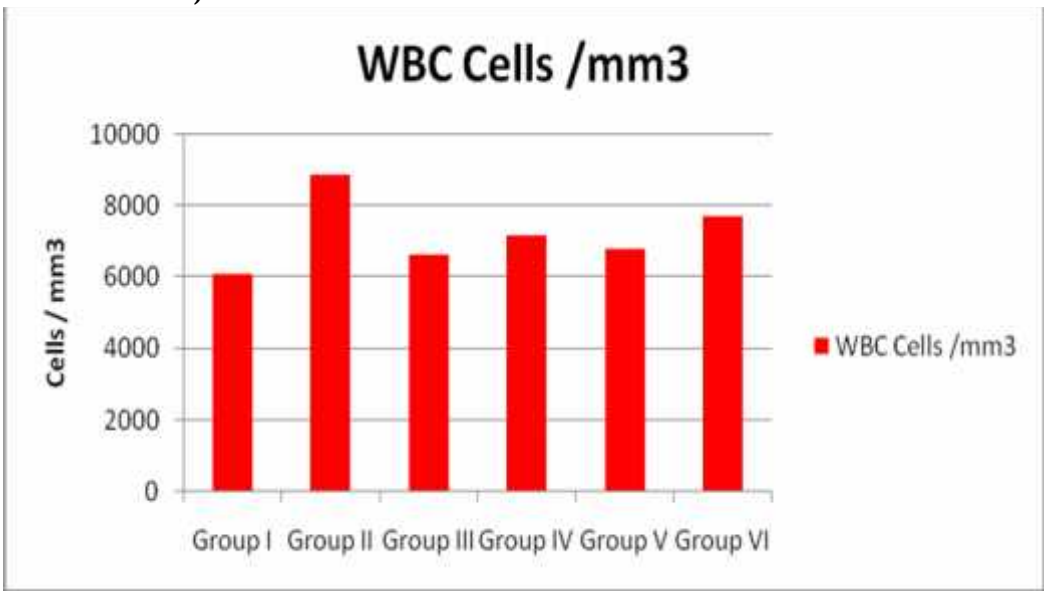
Estimation of WBC and differential count

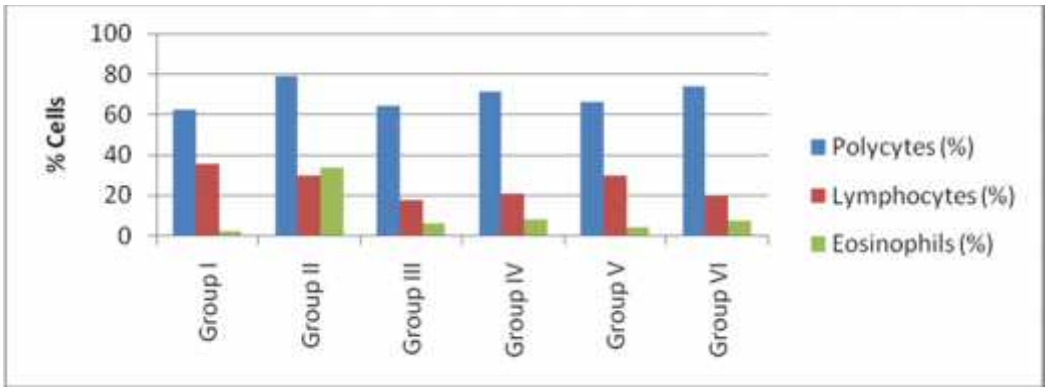
Table 1 : The effect of the various dried root extract of *Desmodium gangeticum* DC (DGCRE, DGERE and DGART) on WBC and differential count

Groups	Treatment	Dose	WBC Cells/mm^3	Polycytes (%)	Lymphocytes (%)	Eosinophils (%)
Group I	Solvent Control-I (Normal)	1ml/kg	6109 ± 27.460***	62.234 ± 0.5661***	35.917 ± 0.8920***	2.4 ± 0.1414***
Group II	Control- II (Sensitized control)	5mg/kg (OVA)	8891.67 ± 47.288	79.267 ± 0.3518	29.834 ± 0.6736	33.67 ± 0.1085
Group III	Standard drug (Ketotifen)	5mg/kg	6625 ± 35.66	64.5 ± 0.5933***	17.861 ± 0.4752***	6.6 ± 0.1145***
Group IV	Treatment-I (DGCRE)	200mg/kg	7178.3±37.006***	71.25 ± 0.2964**	20.8 ± 0.5621*	8.434 ± 0.0954***
Group V	Treatment-II (DGERT)	200mg/kg	6815 ± 37.394***	66.3 ± 0.5304***	29.917 ± 0.5683	4.417 ± 0.1424***
Group VI	Treatment-III (DGART)	200mg/kg	7710 ± 34.254***	74.1 ± 0.5814**	19.9 ± 0.7484^{ns}	7.517 ± 0.1014***

Values are mean ± SEM; n=6 number of animals in each group ***P< 0.001 when compared to all group with control-II **P< 0.01 when compared to all group with control –II *P< 0.05 when compared to all group with control-II ^{ns}P> 0.05 when compared to all group with control-II
Followed by one way ANOVA (analysis of variance) and Bonferroni multiple comparisons test
DGCRT**: -** dried root chloroform extract of *Desmodium gangeticum* DC
DGERT**: -**dried root ethanolic extract *Desmodium gangeticum* DC
DGART**: -**dried root hydro-alcoholic extract *Desmodium gangeticum* DC

Figure 1 : The effect of the dried root extract *Desmodium gangeticum* DC (DGCRE, DGERE and DGART) on WBC and differential count





In the estimation of differential WBC counting, the following values were observed. For control II (sensitized control) animals, the total WBC count was found to be 8891.67±47.288 cells /mm^3. The (normal animal) solvent control I, which received only the saline showed the value of 6109±27.460 cells /mm^3 (P<0.001). The animals treated with Ketotifen showed the value of 6625±35.66 cells /mm^3 (P<0.001), whereas the animals treated with *Desmodium gangitecum* DC chloroform root extract, *Desmodium gangitecum* DC ethanolic root extract and *Desmodium gangitecum* DC hydro alcoholic root extract showed the values of 7178.334±37.006 cells /mm^3 (P<0.001),6815±37.394 and 7710±34.254 cells /mm^3 (P<0.01) respectively. The above values showed that the treatment with *Desmodium gangitecum* DC chloroform root extract, *Desmodium gangeticum* DC ethanolic root extract and *Desmodium gangitecum* DC hydro alcoholic root extracts significantly reduced WBC count as compared to that of control II (sensitized control)

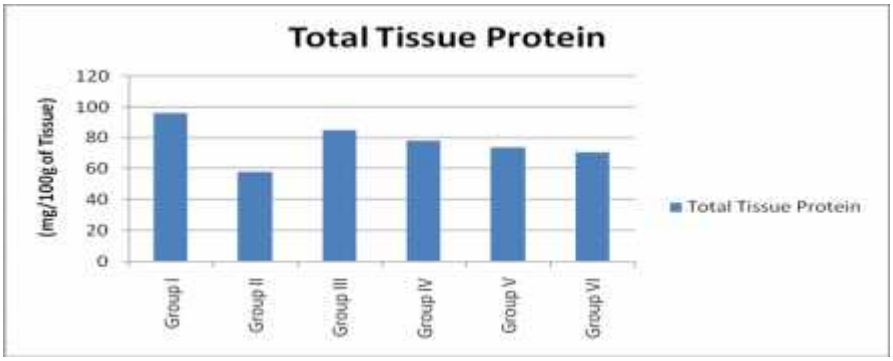
Estimation of Total protein from lung tissue homogenate :

Table 2 : The Total tissue protein from the lung tissue homeogenate obtained from the various dried root extracts of chloroform, ethanol and hydro-alcoholic extracts of *Desmodium gangitecum* DC treated rats.

Groups	Treatment groups	Dose	Total tissue protein (mg/100gm of tissue)
I	Solvent Control-I (Normal)	1ml/kg	96.04±0.5889 ***
II	Control-II(Sensitized control)	5mg/kg	58.262±0.5525
III	Standard drug Ketotifen	5mg/kg	85.145±0.5901***
IV	Treatment-I (DGCRE)	200mg/kg	78.037±0.5780***
V	Treatment-II (DGERT)	200mg/kg	74.029±1.029***
VI	Treatment-II (DGHRT)	200mg/kg	71.029±0.5087***

Values are mean (n=6) ***P<0.001 when compared to all groups with control II followed by one way ANOVA (analysis of variance) and Bonferroni multiple comparison test.

Figure 2 : The Total tissue protein from the lung tissue homeogenate obtained from the various dried root extracts of chloroform, ethanol and hydro- alcoholic extracts of *Desmodium gangitecum* DC treated rats.



The total tissue protein in the control II group (sensitized control) was found to be 58.262 ±0.5525. The (solvent normal) control I, which received only the solvent, had the value of 96.04 ±o.5889 ($p<0.001$). The animals treated with Ketotifen showed the value of 85.145±0.5901 ($P<0.001$). Whereas animals treated with *Desmodium gangitecum* DC chloroform root extract, *Desmodium gangitecum* DC ethanolic root extract and *Desmodium gangitecum* DC hydro-alcoholic root extract were at a dose of 200 mg/kg and had showed the value of 78.037±0.5780 ($p>0.001$),74.029±1.029 and 71.029±0.5087 ($P>0.001$) respectively. The above values showed that *Desmodium gangitecum* DC Chloroform root extract treatment retained the normal total protein level in the lung tissue of experimental rats.

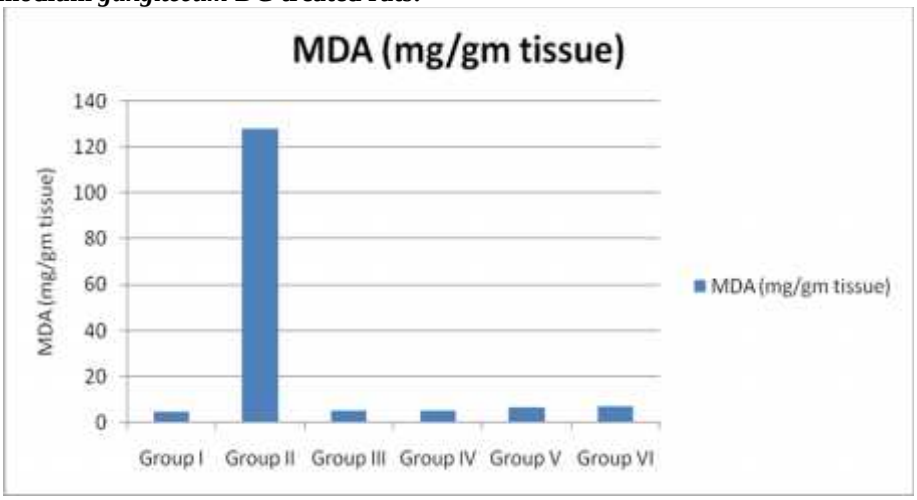
Estimation of tissue Malonyldialdehyde (MDA) level

Table 3: The estimation of Malonyldialdehyde from the dried root extacts of chloroform, ethanol and hydro-alcoholic from *Desmodium gangitecum* DC treated rats:-

Groups	Treatment groups	Dose	MDA (mg/gm tissue)
Group I	Solvent Control-I (Normal)	1ml/kg	4.695±0.2037***
Group II	Control-II (Sensitized control)	5mg/kg	127.955±0.6779
Group III	Standard drug Ketotifen	5mg/kg	5.067±0.1040***
Group IV	Treatment-I (DGCRE)	200mg/kg	5.165±0.1386***
Group V	Treatment-II (DGERT)	200mg/kg	6.739±0.1266***
Group VI	Treatment-II (DGHRT)	200mg/kg	7.157±0.09622***

Values are mean (n=6) ***P < 0.001 when compared to all group with control II followed by one way ANOVA (analysis of variance) and Bonferroni multiple comparison test.

Figure 3: The estimation of Malonyldialdehyde from the dried root extracts of chloroform, ethanol and hydro-alcoholic from Desmodium *gangitecum* DC treated rats:-



The tissue malonyldialdehyde (MDA) level in the control II group (sensitized control) was found to be 127.955±10.6779. The solvent control I, which received only the solvent, had the value of 4.695±0.2037 ($p<0.001$). The animals treated with Ketotifen showed the value of 5.067±0.1040 ($P<0.001$) where as animals treated with *Desmodium gangitecum* DC Chloroform root extract, *Desmodium gangitecum* DC ethanolic root extract and *Desmodium gangitecum* DC hydroalcoholic root extract were at a dose of 200 mg/kg body weight and had showed the value of 5.165± 0.1386 ($p<0.001$), 6.739±0.1266 ($P<0.001$) and 7.157±0.09622 ($P<0.001$) respectively. The above values showed that *Desmodium gangitecum* DC chloroform root extract, *Desmodium gangitecum* DC ethanolic root extract and *Desmodium gangitecum* DC hydroalcoholic root extract treatment normalizes the increased tissue MDA level in experimental rats.

Discussion

The dried roots of *Desmodium gangitecum* DC were collected with the help of *Mullukurumbas* tribal medical practioners of Gudalur, The Nilgiris and aunthenticated. The dried roots were powdered. The dried root powder of *Desmodium gangitecum* DC was extracted with chloroform, ethanol and aqueous alcohol.

The extracts were screened for their preliminary qualitative phytochemical analysis[15] and anti-asthmatic activity. The preliminary phytochemical analysis revealed that the dried root chloroform, ethanolic and aqueous alcoholic extracts of the plant *Desmodium gangitecum* DC were screened for their preliminary phytochemical analysis, in which it showed the presence of alkaloids, carbohydrates, glycosides, phytosterol and flavonoids. The various dried extracts obtained from the Desmodium gangitecum DC were subjected for the anti-asthmatic activity by ovalbumin induced Wister rats showed a significant anti-asthmatic activity in the various parameters estimated for WBC and differential count, Total tissue protein from the lung tissue homogenate and Malonyldialdehyde

Conclusion

The plant had been investigated in a systemic way covering its phytochemical and anti-asthmatic aspects to rationalize its use as a drug. In this present study, the anti-asthmatic effects of the chloroform, ethanolic and hydro-alcoholic dried root extracts of *Desmodium gangitecum* DC were evaluated. There was a significant decrease in WBC count for extract-treated rats as compared to sensitized control II (Ovalbumin) treated rats and the significance was observed for the estimation of total tissue protein content as compared to sensitized control II treated rats. The extracts showed significant results of tissue Malonyldialdehyd**e (**MDA) levels in the experimental rats as compared to sensitized control II treated rats and this study indicates that the veracity of anti-asthmatic activity claimed by the natural medical practitioners of The Nilgiris.

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