

Effect of *Cephalandra indica* against advanced glycation end products, sorbitol accumulation and aldose reductase activity in homoeopathic formulation

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Abstract

Background: Extreme generation of free radicals leads to oxidative stress which has been apprehensive in several disease processes such as diabetic complications and vascular and neurodegenerative diseases. **Objective:** The present study was designed to evaluate the potential of homoeopathic preparations of *Cephalandra indica* L. against oxidative stress. **Materials and Methods:** Potencies of *Cephalandra indica* (mother tincture, 6C and 30C) were procured from Dr. Willmar Schwabe India Pvt. Ltd. The antioxidant activity of *Cephalandra indica* was evaluated by employing various *in vitro* antioxidant methods. **Results:** The total phenol content was found to be 1905, 849 and 495 mg/g gallic acid equivalents in mother tincture, 6C and 30C of *Cephalandra indica* and total antioxidant capacity was found to be 2710, 759 and 510 μ M/g ascorbic acid equivalents, respectively. Mother tincture, 6C and 30C of *Cephalandra indica* was found to have strong reducing power, 2,2-diphenyl-1-picrylhydrazyl radical, hydrogen peroxide, nitric oxide and superoxide radical scavenging activity. Percentage inhibition of AGEs formation by mother tincture, 6C and 30C of *Cephalandra indica* (10–50 μ l) was found to be 30.34%–91.77%, 29.98%–65.71% and 33.05%–57.75%, respectively. Mother tincture, 6C and 30C of *Cephalandra indica* showed inhibitory effect against sorbitol accumulation with IC_{50} value of 26.12 μ l, 203.10 μ l and 897.3 μ l, respectively, whereas, in aldose reductase inhibition assay, the IC_{50} value was 32.54 μ l, 175.02 μ l and 834.34 μ l, respectively. **Conclusion:** The results revealed that homoeopathic preparations of *Cephalandra indica* exhibit protective effect against oxidative stress.

Keywords: Advanced glycation end products, Aldose reductase inhibition, *Cephalandra indica*, diabetic complications, Homoeopathic preparations, Oxidative stress, Sorbitol accumulation

INTRODUCTION

Generation of free radicals and reactive oxygen species (ROS) leads to the oxidative damage to cellular biomolecules such as proteins, lipids and DNA which is considered to play an important role in the prevalence of several chronic diseases.^[1,2]

Cephalandra indica belongs to family *Cucurbitaceae*, also known as Kundru in Hindi and Ivy Gourd in English, is a creeper vegetable that grows wild and in abundance in major parts of India. It is a perennial climbing herb with tuberous roots, fruiting throughout the year. The plant has been used since ancient times for treating Diabetes Mellitus in the Indian system of medicine known as Ayurveda. As it was significantly effective in diabetes treatment, *Cephalandra indica* was described by some as 'Indian Substitute for Insulin'.^[3] The plant also gained many reported scientific values as antidiabetic

medicine.^[4,5] Fresh juice of roots is used to treat diabetes; tincture of leaves is used to treat gonorrhoea and paste of leaves is applied to the skin diseases. Dried bark is a good cathartic. Leaves and stem are antispasmodic and expectorant. The fleshy green fruit is very bitter. Green fruit is chewed to cure sores on the tongue.^[6,7]

In the present study, *in vitro* antioxidative potential of mother tincture, 6C and 30C potencies of *Cephalandra indica* was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free

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radical scavenging assay, nitric oxide (NO) scavenging assay, hydrogen peroxide scavenging assay, reducing power, superoxide radical scavenging activity, total phenolic content by Folin-Ciocalteu method and total antioxidant activity by phosphomolybdenum method.

MATERIALS AND METHODS

Materials and reagents

Potencies (Mother tincture, 6C and 30C) of *Cephalandra indica* L. were procured from Dr. Willmar Schwabe India Pvt. Ltd. DPPH and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid, bovine serum albumin (BSA), nitroblue tetrazolium (NBT), Folin-Ciocalteu reagent and *N*-(1-Naphthyl) ethylenediamine dihydrochloride were purchased from Molychem Pvt. Ltd., India. All other chemicals and reagents used were of analytical grade. Study protocol was approved and conducted in MMCP, Maharishi Markandeshwar University, Mullana, Ambala, Haryana, India.

In vitro antioxidant activity

Determination of total phenol content

Total phenolic content in the mother tincture, 6C and 30C of *Cephalandra indica* was determined with Folin-Ciocalteu reagent using gallic acid as a standard phenolic compound. Sample was diluted appropriately to obtain absorbance in the range of calibration curve. An aliquot of 1 ml of sample solution was mixed with 1 ml of Folin-Ciocalteu reagent. Three minutes later, 3.0 ml of 2% sodium carbonate was added, and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue colour that developed was measured at 760 nm (UV-VIS spectrophotometer - 1800, Shimadzu, Japan). The concentration of total phenolic compounds in the sample was obtained as milligrams of gallic acid equivalent (GAE) per gram dry weight.^[8]

Total antioxidant capacity

An aliquot of 0.3 ml of mother tincture, 6C and 30C of *Cephalandra indica* was mixed with 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank, 0.3 ml of water was used instead of sample. The tubes were capped with aluminium foil and incubated in boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of ascorbic acid ($\mu\text{mol/g}$).^[9]

2,2-diphenyl-1-picrylhydrazyl scavenging activity

The DPPH radical scavenging ability of mother tincture, 6C and 30C of *Cephalandra indica* was evaluated according to the method given in literature with slight modification.^[10] The different concentrations in each reaction set were mixed with 1.0 ml of 0.1 mM of DPPH in ethanol. The mixture was incubated in the dark for 30 min at room temperature. Degree of inhibition of DPPH by monitoring the decrease in

absorbance measured at 517 nm. Ascorbic acid was used as positive control. Radical scavenging activity was expressed as inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was the absorbance of control (blank without sample) and A_t was the absorbance in presence of sample. All the tests were performed in triplicate and graph was plotted with mean values.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity was evaluated as described previously.^[11] An aliquot of 40 mM H_2O_2 solution (0.6 ml) was mixed with various concentrations of mother tincture, 6C and 30C of *Cephalandra indica*. To the mixture, 2.4 ml of phosphate buffer (0.1 M, pH 7.4) was added and the mixture was shaken vigorously and incubated at room temperature for 10 min. Then, the absorbance of the reaction mixture was determined at 230 nm. Ascorbic acid was used as positive control. The H_2O_2 scavenging activity was calculated as follows:

$$\% \text{ Inhibition} = 1 - \left(\frac{A_1 - A_2}{A_0} \right) \times 100$$

Where A_0 is the absorbance of the control (water instead of sample), A_1 is the absorbance of the sample and A_2 is the absorbance of the sample only (phosphate buffer instead of H_2O_2 solution). The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of H_2O_2 .

Reducing power assay

The Fe^{3+} -reducing power of mother tincture, 6C and 30C of *Cephalandra indica* was determined according to the method described in literature.^[12] Different concentrations of samples (2.5 ml) were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 1000 rpm for 8 min. The supernatant (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The assay was carried out in triplicate and the results expressed as mean values \pm standard deviations. Ascorbic acid was used as positive control. The sample concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph plotted between absorbance at 700 nm against sample concentration.

Nitric oxide scavenging activity

At physiological pH, aqueous solution of sodium nitroprusside spontaneously generates $\text{NO}^{[13]}$ which interacts with oxygen to produce nitric ions that can be estimated using Griess reagent. Scavengers of NO compete with oxygen, leading to reduce the production of NO. The reaction mixture of 5 mM sodium nitroprusside in phosphate buffer saline (PBS) and 3.0 ml of different concentrations of the mother tincture, 6C and 30C of *Cephalandra*

indica was incubated at 25°C for 150 min. After incubation, the samples were added to Greiss reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthyl ethylenediamine dihydrochloride). The pink chromophore generated during the diazotisation of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine was measured at 546 nm. Ascorbic acid was used as positive control. The percentage of inhibition was measured by the following formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A₀ was the absorbance of the control (blank, without sample) and A_t was the absorbance in the presence of the sample. All the tests were performed in triplicate and the graph was plotted with the mean values.

Superoxide radical scavenging activity

The activity was measured by the reduction of NBT reagent method as described by Shukla *et al.*, 2009.^[14] The method is based on the generation of superoxide radical (O²⁻) by auto-oxidation of hydroxylamine hydrochloride in the presence of NBT, which gets reduced to nitrite. Nitrite in the presence of ethylenediaminetetraacetic acid (EDTA) gives a colour that was measured at 560 nm. Different concentrations of mother tincture, 6C and 30C of *Cephalandra indica* were taken in a test tube. To this, reaction mixture consisting of 1 ml of (50 mM) sodium carbonate, 0.4 ml of (24 mM) NBT and 0.2 ml of 0.1 mM EDTA solutions were added to the test tube and immediate reading was taken at 560 nm. After incubating the reaction mixture at 25°C for 15 min, about 0.4 ml of (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction and reduction of NBT was measured at 560 nm. Ascorbic acid was used as the positive control. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage of inhibition was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A₀ was the absorbance of the control (blank, without sample) and A_t was the absorbance in the presence of the samples. All the tests were performed in triplicate and the graph was plotted with the mean values.

Antiglycation activity

In vitro antiglycation activity of mother tincture, 6C and 30C of *Cephalandra indica* was examined by testing their ability to inhibit the fluorescence of BSA in accordance with a previous method.^[15] The reaction mixture of BSA (10 mg/ml), 1.1 M fructose in 0.1 M PBS and pH 7.4 containing 0.02% sodium azide with or without sample (Mother tincture, 6C and 30C of *Cephalandra indica* dissolved in PBS) was incubated in darkness at 37°C for 1, 2, 3 and 4 weeks. AGE formation was measured by fluorescent intensity at an excitation wavelength 355 nm and emission wavelength 460 nm using Elico-SLI74

spectrofluorometer fitted with Xenon Lamp (Elico, India). Aminoguanidine (AG; 500 µg/ml) was used as a positive control for this study.

Erythrocyte sorbitol accumulation inhibition

Five millilitres of heparinised blood was collected from overnight fasted healthy male Wistar rats and erythrocytes were separated from the plasma by centrifugation at 3000 g for 30 min. The cells were washed three times with isotonic saline at 4°C, and in the final washing, the cells were centrifuged at 1500 g for 15 min to obtain a consistently packed cell preparation. The packed cells (1 mL) were then incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) (4 mL) containing 55 mM glucose in the presence or absence of samples (Mother tincture, 6C and 30C of *Cephalandra indica*) at 37°C for 3 h. The erythrocytes were washed with cold saline by centrifugation at 2000 g for 5 min, precipitated by adding 6% of cold perchloric acid (3 mL) and centrifuged again at 2000 g for 10 min. The supernatant was neutralised with 2.5 M K₂CO₃ at 4°C and used for sorbitol determination.^[16] The relative fluorescence due to NADH was measured by a fluorescence spectrometer (Elico-SLI74 spectrofluorometer fitted with Xenon Lamp, Elico, India) at an excitation wavelength of 366 nm and an emission wavelength of 452 nm. The experiments were performed in triplicates.

Aldose reductase enzyme inhibition

Partial purification of aldose reductase (ALR1) from rat kidney (IAEC Protocol No: MMCP/IAEC/13/07) was carried out following the previously described methods.^[17] Isolated kidney was homogenised in 3 volumes of 10 mM sodium phosphate buffer, pH 7.2 containing 0.25 M sucrose, 2.0 mM EDTA and 2.5 mM 2-mercaptoethanol. The homogenate was centrifuged at 10,000 g for 20 min and the supernatant was subjected to ammonium sulphate precipitation. Precipitate obtained between 45% and 75% saturation was dissolved in the above buffer. The supernatant was used as the source of ALR1. The activity of ALR1 was measured spectrophotometrically by monitoring the oxidation of NADPH at 340 nm as a function of time at 37°C using glyceraldehyde as substrate. The assay mixture in 1 ml contained 50 mM sodium phosphate buffer of pH 7.2, 0.2 M ammonium sulphate, 10 mM DL-glyceraldehyde, 5 mM β-mercaptoethanol and 0.1 mM NADPH. Various concentrations of mother tincture, 6C and 30C of *Cephalandra indica* were added to assay mixtures of ALR1 and incubated for 5 min before initiating the reaction by NADPH as described above. The percentage inhibition was calculated considering the activity in the absence of mother tincture, 6C and 30C of *Cephalandra indica* as 100%. The IC₅₀ values were determined by linear regression analysis of the plot of percentage inhibition versus inhibitor concentration.

RESULTS AND DISCUSSION

Total phenol content

Phenols and polyphenols are the main class of natural antioxidants found in plants whose functions is to strengthen

the oxidative stability of foods and human systems due to their redox properties, which plays significant role in neutralising free radicals, quenching singlet oxygen or decomposing hydroperoxides.^[18,19] Total phenol content in homoeopathic preparations of *Cephalandra indica* was determined by Folin–Ciocalteu reagent. The total phenol content was found to be 1905, 849 and 495 mg/g GAEs in mother tincture, 6C and 30C of *Cephalandra indica*, respectively.

Total antioxidant capacity

The total antioxidant capacity is used for the evaluation of both water- and fat-soluble antioxidants. The phosphomolybdenum method used for measuring total antioxidant capacity is based on the formation of green phosphate/reduced Mo (V) complex at acidic pH.^[20] Total antioxidant capacity of mother tincture, 6C and 30C of *Cephalandra indica* was found to be 2710, 759 and 501 $\mu\text{M/g}$ ascorbic acid equivalent, respectively.

2,2-diphenyl-1-picrylhydrazyl scavenging activity

Assay based on the use of DPPH radicals is the most popular spectrophotometric methods for the determination of the antioxidant capacity of plant extracts, foods, beverages and vegetable extracts because the radical compounds can directly react with antioxidants.^[21] The present results suggest that mother tincture, 6C and 30C of *Cephalandra indica* is apparently good free radical scavengers. The IC_{50} value of mother tincture, 6C and 30C of *Cephalandra indica* was 28.23, 155.14 and 923.02 μl respectively [Figure 1] and that of ascorbic acid was found to be 1.43 $\mu\text{g/ml}$.

Hydrogen peroxide scavenging activity

H_2O_2 is considered as one of the main inducers of cellular ageing and could strike many cellular energy-producing systems.^[22] H_2O_2 is an uncharged species and is not very reactive itself. It penetrates in cellular membrane and leads to formation of hydroxyl radical which the capability of damaging almost every molecule found in the living cells.^[23] The IC_{50} value of mother tincture, 6C and 30C of *Cephalandra indica* was found to be 32.52 μl , 185.14 μl and 939.05 μl ml, respectively [Figure 2]. IC_{50} value of ascorbic was found to be 80 $\mu\text{g/ml}$.

Reducing power assay

The electron donating capacity reflects the reducing power of bioactive compounds and is associated with antioxidant activity. Antioxidants can be reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other. The presence of reductants, such as antioxidant substances in the samples, causes the reduction of the $\text{Fe}^{3+}/\text{ferricyanide}$ complex to the ferrous form.^[24] The formation of ferrous ion complex was observed and EC_{50} (effective concentration at which the absorbance is 0.5) was calculated [Figure 3]. EC_{50} was found to be 37 μl , 250 μl and 989 μl for mother tincture, 6C and 30C of *Cephalandra indica*, respectively, and 21.42 $\mu\text{g/ml}$ for ascorbic acid.

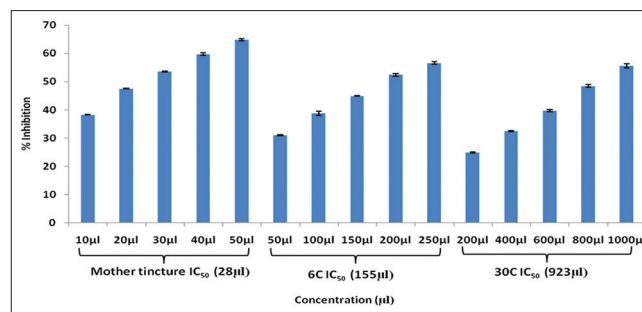


Figure 1: Effect of *Cephalandra indica* on 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities in homoeopathic formulation. Values are mean \pm standard deviation for $n = 3$

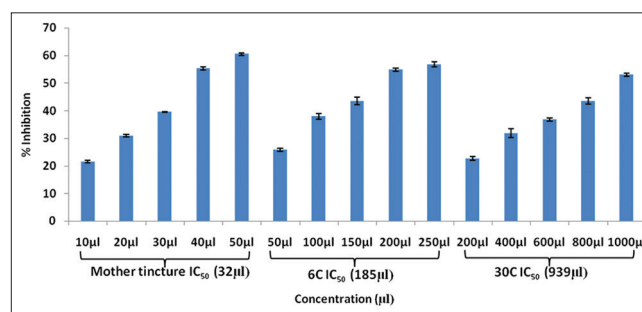


Figure 2: Effect of *Cephalandra indica* on H_2O_2 radical scavenging activities in homoeopathic formulation. Values are mean \pm standard deviation for $n = 3$

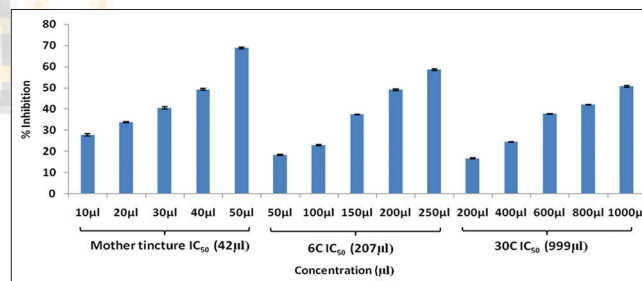


Figure 3: Effect of *Cephalandra indica* on reducing power in homoeopathic formulation. Values are mean \pm standard deviation for $n = 3$

Nitric oxide scavenging activity

NO is generated from amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells of the brain. NO is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion.^[25] The various concentrations of mother tincture, 6C and 30C of *Cephalandra indica* showed significant inhibition against NO radical in a dose-dependent manner. The concentration of mother tincture, 6C and 30C of *Cephalandra indica* required for 50% inhibition (IC_{50}) was found to be 42.13 μl , 207.05 μl and 999 μl , respectively [Figure 4]. IC_{50} of ascorbic acid was found to be 63.55 $\mu\text{g/ml}$.

Superoxide scavenging activity

Superoxide anion radicals are formed due to electron leakage from electron transport chain in aerobic cells and acts as a precursor for ROS that contributes to tissue damage and various other pathological conditions.^[26] Mother tincture, 6C and 30C of *Cephalandra indica*, had significant activity against superoxide radicals in a dose-dependent manner [Figure 5]. IC₅₀ of ascorbic acid was found to be 27.96 µg/ml and that of mother tincture, 6C and 30C of *Cephalandra indica* was found to be 33.65 µl, 223 µl and 987 µl, respectively.

AGEs inhibition activity

Hyperglycaemia leads to the production of AGEs and their receptors. AGEs are responsible for production of ROS and thus results to oxidative stress, the major element for onset of diabetic complications.^[27] In the present study, the formation of AGEs was monitored weekly by measuring fluorescence intensity of the BSA-fructose solutions for 4 weeks and mother tincture, 6C and 30C, potencies of *Cephalandra indica* were found to have inhibitory effect against the formation of AGEs. A significant inhibition of AGEs formation (93.37%) was observed in fructose-induced glycated BSA plus AG (500 µg/ml). At 4th week of incubation, the percentage inhibitions of AGEs formation by *Cephalandra indica* mother tincture (10–50 µl) were 30.34%–91.77%, respectively; 6C (50–250 µl) was 29.98%–65.71% and for 30C (200–1000 µl) was found to be 33.05%–57.75%, respectively [Figures 6-8].

Erythrocyte sorbitol accumulation inhibition

The excessive formation and accumulation of sorbitol during chronic hyperglycaemia lead to increased oxidative stress and ultimately to diabetic complications.^[28] The different potencies of *Cephalandra indica* in the present study were found to exhibit significant inhibitory effect on accumulation of sorbitol in red blood cells [Figure 9]. IC₅₀ of ascorbic acid was found to be 183.08 µg/ml and that of mother tincture, 6C and 30C of *Cephalandra indica* was found to be 26.23 µl, 203.14 µl and 897.54 µl, respectively.

Aldose reductase inhibitory activity

Chronic hyperglycaemia is responsible for the activation of polyol pathway through the enzyme aldose reductase. The increased activity of this pathway leads to excess formation of sorbitol.^[28] The enzyme ALR1 was obtained from kidney of Wistar rat and the activity of ALR1 was measured spectrophotometrically by monitoring the oxidation of NADPH at 340 nm. IC₅₀ of quercetin (standard) was found to be 5.30 µg/ml and that of mother tincture, 6C and 30C of *Cephalandra indica* was found to be 32.19 µl, 175.01 µl and 834 µl respectively [Figure 10].

CONCLUSION

Free radicals produced from different physiological and non-physiological processes are one of the bases of

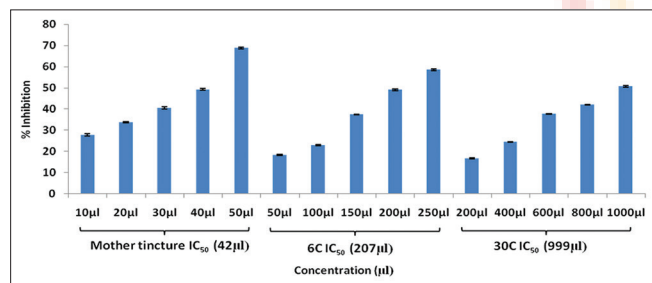


Figure 4: Effect of *Cephalandra indica* on NO radical scavenging activities in homoeopathic formulation. Values are mean ± standard deviation for n = 3

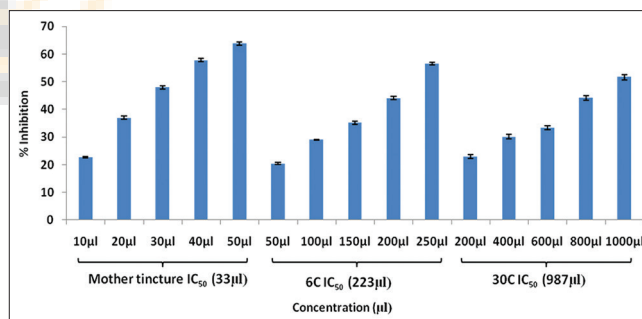


Figure 5: Effect of *Cephalandra indica* on SOD radical scavenging activities in homoeopathic formulation. Values are mean ± standard deviation for n = 3

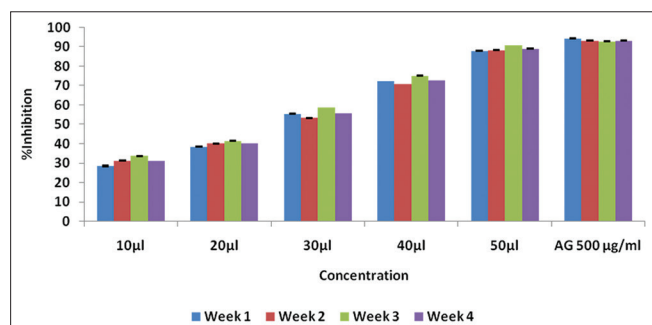


Figure 6: The effects of *Cephalandra indica* mother tincture on the formation of fluorescent advanced glycation end products in bovine serum albumin incubated with fructose. Values are mean ± standard deviation for n = 3. AG: Aminoguanidine

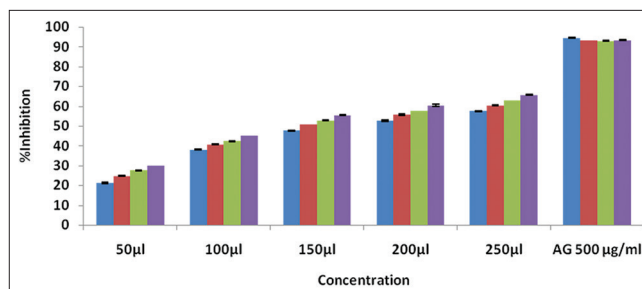


Figure 7: The effects of *Cephalandra indica* 6C on formation of fluorescent advanced glycation end products in bovine serum albumin incubated with fructose. Values are mean ± standard deviation for n = 3. AG: Aminoguanidine

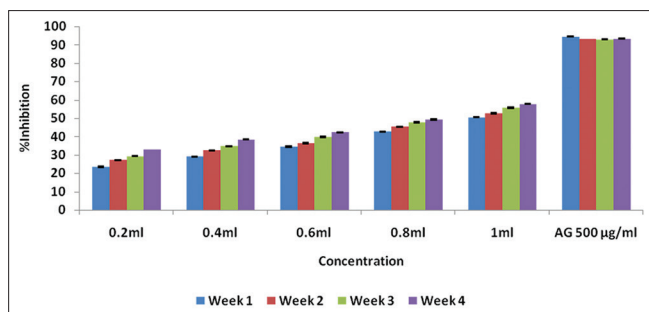


Figure 8: The effects of *Cephalandra indica* 30C on the formation of fluorescent advanced glycation end products in bovine serum albumin incubated with fructose. Values are mean \pm standard deviation for $n = 3$. AG: Aminoguanidine

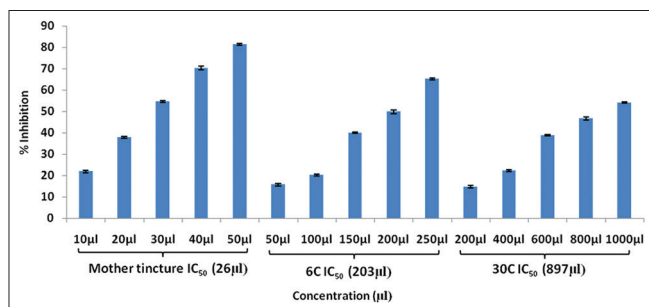


Figure 9: Effect of *Cephalandra indica* on erythrocyte sorbitol accumulation inhibition assay in homoeopathic formulation. Values are mean \pm standard deviation for $n = 3$

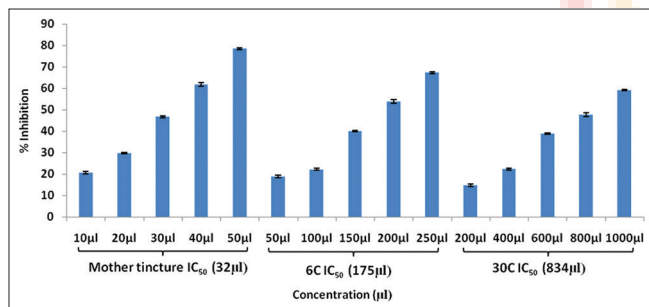


Figure 10: Effect of *Cephalandra indica* on aldose reductase inhibitory assay in homoeopathic formulation. Values are mean \pm standard deviation for $n = 3$

several diseases. Therefore, there is an urge of safe and potent antioxidants to overthrow the free radicals. The results from the present study revealed the presence of phenols in homoeopathic preparation of *Cephalandra indica* which plays a significant role in the reduction of oxidative stress. *Cephalandra indica* was found to scavenge different radicals such as in DPPH, H_2O_2 , NO and SOD. *Cephalandra indica* was also found to inhibit the formation of AGEs and sorbitol accumulation, major culprits for onset of diabetic complications. These defensive effects of homoeopathic preparation of *Cephalandra indica* may suggest its use for the attenuation of oxidative stress and diabetic complications.

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Nil.

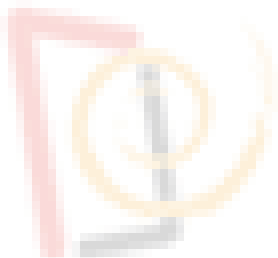
Conflicts of interest

There are no conflicts of interest.

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उन्नत ग्लाइकेशन अंतिम उत्पाद, स्रोबीटोल संचय और होम्योपैथिक फॉर्मूलेशन में अल्डोस रिडक्टेस गतिविधि के विरुद्ध सिफलेंड्रा इंडिका का प्रभाव सार

पृष्ठभूमि: मुक्त कणों का अत्याधिक उत्पादन ऑक्सीडेटिव तनाव की ओर ले जाता है जोकि मधुमेह जटिलताएं, संवहनी और न्यूरोडिजेनेरेटिव रोगों आदि जैसे रोगों में संदेही रहा है।

उद्देश्य: प्रस्तुत अध्ययन ऑक्सीडेटिव तनाव के विरुद्ध होम्योपैथिक औषधि सिफलेंड्रा इंडिका एल. की क्षमता का मूल्यांकन करने के लिए तैयार किया गया।

सामग्री व विधि: सी. इंडिका की पोटेन्सी (मदर टिंक्चर, 6सी और 30सी) डॉ. विल्मर श्वाबे इंडिया प्राइवेट लिमिटेड से ली गई। इन-विट्रो एंटीऑक्सीडेंट तरीकों से सी. इंडिका की एंटीऑक्सीडेंट गतिविधियों का मूल्यांकन किया गया।

परिणाम: कुल फेनोल मात्रा 1905, 849 और 495 मिलीग्राम/ग्राम मेंगैलिक एसिड समतुल्य, सी. इंडिका के मदर टिंक्चर 6सी और 30सी और कुल एंटीऑक्सीडेंट क्षमता 2710 और 759 व 510 माइक्रोग्राम/ग्राम अर्साबिक एसिड समतुल्य पाई गई। सी. इंडिका की 6सी और 30सी और मदर टिंक्चर में रिड्यूसिंग पावर, डीपीएच कण, हाइड्रोजन पेरोक्साइड, नाइट्रिक ऑक्साइड और सुपर ऑक्साइड कण को स्वच्छ करने की क्षमता पाई गई। सी. इंडिका (10–50 μl) के 6सी एवं 30सी मदर टिंक्चर के द्वारा अग्रिम ग्लाइकेशन अंतिम उत्पाद का प्रतिशत निषेध क्रमशः 30.34 से 91.77 प्रतिशत 29.98 से 65.71 और 33.05 से 57.75 प्रतिशत पाया गया। सी. इंडिका के मदर टिंक्चर, 6सी और 30सी पोटेन्सी के सोरबिटोल संचय के विरुद्ध निरोध तात्क प्रभाव आईसी50 मात्रा के साथ क्रमशः 26.12 μl , 203.10 μl और 897.3 μl पाये गये, जबकि अल्डोस रिडक्टेज निषेध मात्रा 32.54 μl का आईसी50 175.02 μl और 834.34 μl पायी गई।

निष्कर्ष: परिणाम दिखाते हैं कि होम्योपैथिक औषधि सिफलेंड्रा इंडिका ऑक्सीडेटिव तनाव के विरुद्ध सुरक्षात्मक प्रभाव प्रदर्शित करती है।

Wirkung von *Cephalandra indica* gegen fortgeschrittene Akkumulation und Aldosereduktaseaktivität in homöopathischer Formulierung.

Abstrakt

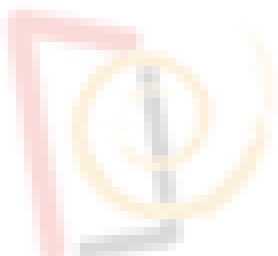
Hintergrund: Extreme Bildung von freien Radikalen führt zu oxidativem Stress, der bei verschiedenen Krankheitsprozessen, wie diabetischen Komplikationen und vaskulären und neurodegenerativen Erkrankungen, befürchtet wurde.

Zielsetzung: Die vorliegende Studie wurde entworfen, um das Potenzial von homöopathischen Präparaten von *Cephalandra indica* L. gegen oxidativen Stress zu bewerten.

Material und Methoden: Potenzen von *Cephalandra indica* (Urtinktur, 6C und 30C) wurden von Dr. Willmar Schwabe India Pvt. Die antioxidative Aktivität von *Cephalandra indica* wurde unter Verwendung verschiedener in vitro-Antioxidationsmethoden bewertet.

Ergebnisse: Es wurde gefunden, dass der Gesamtphenolgehalt 1905, 849 und 495 mg / g Gallussäureäquivalente in der Urtinktur, 6C und 30C *Cephalandra indica* betrug und dass die gesamte antioxidative Kapazität 2710, 759 und 510 µM / g Ascorbinsäureäquivalente betrug, beziehungsweise. Es wurde gefunden, daß die Urtinktur, 6C und 30C von *Cephalandra indica* starke Reduktionskraft, 2,2-Diphenyl-1-picrylhydrazyl-Radikal, Wasserstoffperoxid, Stickoxid und Superoxid-Radikalfängeraktivität aufwiesen. Die prozentuale Hemmung der Bildung von AGEs durch Urtinktur, 6C und 30C *Cephalandra indica* (10-50 & mgr; l) betrug 30,34% -91,77%, 29,98% -65,71% bzw. 33,05% -57,75%. Die Urtinktur, 6C und 30C von *Cephalandra indica*, zeigten eine inhibitorische Wirkung gegen die Sorbitakkumulation mit einem IC₅₀-Wert von 26,12 & mgr; l, 203,8 & mgr; l bzw. 897,3 & mgr; l, während der IC₅₀-Wert im Aldosereduktase-Inhibitionstest 32,54 & mgr; l, 175,02 & mgr; l und 834,34 & mgr; l beziehungsweise.

Fazit: Die Ergebnisse zeigten, dass homöopathische Zubereitungen von *Cephalandra indica* eine schützende Wirkung gegen oxidativen Stress zeigen.



Efecto de la formulación homeopática de *Cephalandra indica* frente a los productos finales de la glicación avanzada, la acumulación de sorbitol y la actividad de la aldosareductasa

Resumen

Fundamento: La generación de radicales libres da lugar a un estrés oxidativo que ha sido problemático en varios procesos patológicos como las complicaciones de la diabetes y las enfermedades vasculares y neurodegenerativas.

Objetivos: El presente estudio se diseñó para evaluar el potencial de los preparados homeopáticos de *Cephalandra indica* L. frente al estrés oxidativo.

Materiales y métodos: El laboratorio Dr. Willmar Schwabe India Pvt. Ltd. suministró las potencias de *Cephalandra indica* (tintura madre, 6C y 30C). La actividad antioxidante de *Cephalandra indica* se evaluó empleando diferentes métodos antioxidantes *in vitro*.

Resultados: En la tintura madre y las potencias de 6C y 30C de *Cephalandra indica*, se determinó el contenido total de fenoles en 1.905, 849 y 495 mg/g de equivalentes del ácido gálico y la capacidad antioxidante total, en 2.710, 759 y 510 µM/g de equivalentes del ácido ascórbico, respectivamente. Se constató que la tintura madre y las potencias de 6C y 30C de *Cephalandra indica* poseen un gran poder de reducción y una actividad scavenger potente contra el radical 2,2-difenil-1-picrilhidrazil, el peróxido de hidrógeno, el óxido nítrico y los radicales superóxido. La inhibición porcentual de la formación de AGE (productos finales de glicación avanzada), que ejercen la tintura madre y las potencias de 6C y 30C de *Cephalandra indica* (10–50 µl), se situó en el 30,34%–91,77%, el 29,98%–65,71% y el 33,05%–57,75%, respectivamente. Las tinturas madre y las potencias de 6C y 30C de *Cephalandra indica* mostraron un efecto inhibitor frente a la acumulación de sorbitol con un valor CI₅₀ de 26,12 µl, 203,10 µl y 897,3 µl, respectivamente, mientras que, en el ensayo de inhibición de la aldosareductasa, el valor CI₅₀ fue de 32,54 µl, 175,02 µl y 834,34 µl, respectivamente.

Conclusiones: Los resultados revelaron que los preparados homeopáticos de *Cephalandra indica* exhiben un efecto protector frente al estrés oxidativo.

Effets du *Cephalandra indica* contre les produits finaux de glycation avancée, l'accumulation de sorbitol et l'activité inhibitrice d'aldose réductase dans la formulation homéopathique.

Résumé

Contexte: L'extrême production de radicaux libres entraîne un stress oxydatif appréhendé dans de nombreux processus de maladies comme dans le cas des complications du diabète et des maladies vasculaires et neuro-dégénératives. Objectif : Cette étude a été élaborée pour évaluer le potentiel des préparations homéopathiques de *Cephalandra indica* L. dans le traitement du stress oxydatif.

Matériels et méthodes: Les dilutions du *Cephalandra indica* (la teinture mère, 6C et 30C) ont été obtenues auprès de la société Dr Willmar Schwabe India Pvt. Ltd. L'activité antioxydante du *Cephalandra indica* a été évaluée en utilisant diverses méthodes antioxydantes *in vitro*.

Résultats: La teneur totale en phénol était de 1905, 849 et 495 mg/g d'équivalents en acide gallique dans la teinture mère 6C et 30C de *Cephalandra indica* et la capacité totale antioxydante était de 2710, 759 et 510 $\mu\text{M/g}$ d'équivalents en acide ascorbique respectivement. La teinture mère, 6C et 30C de *Cephalandra indica* semble avoir un fort pouvoir réducteur et une activité piégeage du radical 2,2-diphényl-1-picrylhydrazyl, du peroxyde d'hydrogène, de l'oxyde nitrique et du radical de superoxyde. Le pourcentage d'inhibition de la formation des AGE par la teinture mère 6C et 30C de *Cephalandra indica* (10–50 μl) se trouvait entre 30,34 % et 91,77 %, 29,98 % et 65,71% et entre 33,05 % et 57,75 % respectivement. La teinture mère 6C et 30C de *Cephalandra indica* a montré un effet inhibiteur contre l'accumulation de sorbitol avec une valeur IC_{50} de 26,12 μl , 203,10 μl et de 897,3 μl , respectivement tandis que dans l'analyse de l'inhibiteur d'aldose réductase, la valeur IC_{50} était de 32,54 μl , 175,02 μl et de 834,34 μl respectivement.

Conclusion: Les résultats montrent que les préparations homéopathiques de *Cephalandra indica* ont un effet protecteur contre le stress oxydatif.



順勢療法形式的紅瓜 (*Cephalandra indica*) 對晚期糖基化終產物、山梨醇積累、和醛糖還原酶活性的影響。

摘要

背景: 極端的自由基產生導致氧化壓力，而氧化壓力在幾種疾病的發展過程中一直備受關注，如：糖尿病併發症、血管和神經組織退化性疾病。

目的: 此研究設計的目的是評估順勢療法製劑紅瓜 (*Cephalandra indica*) 對抗氧化壓力的潛力。

材料和方法: 從威爾瑪施瓦布博士印度分公司取得紅瓜的不同層級 (母酊, 6C和30C)。通過採用不同的試管內抗氧化方法來評估紅瓜的抗氧化活性。

結果: 紅瓜的母酊、6C和30C的總酚含量分別為每克1905、849和495毫克的沒食子酸等價物，而總抗氧化能力分別為每克2710、759及510微莫耳抗壞血酸等價物。紅瓜母酊、6C和30C的還原能力、DPPH自由基、過氧化氫、一氧化氮、超氧陰離子自由基的清除能力都很強。紅瓜 (10 – 50 μl) 的母酊、6C和30C抑制AGEs形成的百分率各自分別為30.34%-91.77%、29.98%-65.71%及33.05%-57.75%。紅瓜的母酊、6C和30C展示出抑制山梨醇積累的 IC_{50} 值分別為26.12 μl ，203.10 μl 和897.3 μl ，然而，醛糖還原酶抑制活性的測定的 IC_{50} 值分別為32.54 μl ，175.02 μl 和834.34 μl 。

結論: 結果顯示，順勢療法製劑紅瓜對氧化壓力有保護作用。