Inhibition activity of mucilage prepared of Salvia hispanica as anti-filamentary and anticancer

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Abstract:

The mucilage of white and black chia seeds were extracted and determining their contents of bioactive compounds such as tannins, glycosides, flavonoids, phenols, and studying their activity as anti-filamentary and anticancer. Results showed that tannins exist in white chia seeds with 35%while it is 38% in black seeds, flavonoids represent 0.98mg/ml in white chia seeds and 1.5 mg/ml for black seeds. Both kinds of seeds showed anti-filamentaryactivity, the inhibition ratio was 86.5% for white seeds and 97.3% for black seeds. Chia black seeds showed anticancer activity and the inhibition of the tumor muscle cell line ratio (GI%) was 34.8% at 50mg/ml concentration and 88.4% at 200mg/ml concentration.

Keywords: Chia seeds, *Salvia hispanica*, mucilage,anti-filamentary, anticancer.

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Introduction:

The *Salvia hispanica* plant, known commercially as the Chia plant, is one of the plants that belong to the Lamiaceae family, and it is one of the annual herbaceous plants that grow to a length of 1 meter,and its opposite leaves are 4-8 cm in length and 3-5 cm in width and are characterized by purple and white flowers arranged in spike clusters at the end of each Stalk (Munoz, 2012).The seeds are usually oval and have a diameter of about 1 mm, and it is small seeds found in multiple colors, including, brown, gray, black, and white spotted(Suri *et al.*, 2016).

The genus Salvia is one of the important species belonging to this family, this genus includes more than 1000 species, and the most important are *S. triloba,S.officinal, and S.hispanic*, which are widespread in all parts of the world, and the plant species belonging to this genus contain many active compounds Biologically speaking, such as Glycosides, Trepans, Saponin, Flavonoids, tannins, alkaloids, phenolic compounds, these compounds have an anti-effect for growing of microorganism (bacteria, molds, yeasts), anti-inflammatory and anti-cancer,and that tests are conducted on these compounds in general and in mucilage of plants which was prepared from chia seedsin particular, it is important to identifying the active and vital basis that leads to the discovery and development of medicinal drugs and the use of these compounds which include secondary metabolites in plants which used in human treatment (Vijay *et al.*, 2014).

Inflammation, or what is known as infectious infections, is defined as the entry of microorganisms, such as bacteria, fungi, or viruses causing the occurrence of

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inflammation in a specific part. This inflammation results in the formation of a network complex of cells and the occurrence of tissue interference as a result of the presence of some chemical, physiological, mechanical, as well as infection factors, inflammation is a source of danger and causes various types of cancers such as lung, stomach and pancreatic cancer (Sandhia *et al.,*2015).

Inflammatory infections are associated with pain, redness, swelling, and sometimes lead to the loss of the inflamed part or organ of its ability to doing its vital functions, and that the molecules that cause inflammation are released from white blood cells and cause inflammation (Abdulkhaleo-Gazeem *et al.*, 2016). The phyto-mucilage prepared from chia seeds which containing active compounds such as flavonoids, glycosides, alkaloids, tannins, phenolic compounds has an effective role in relieving infections and had a positive effect against prostate cancer compared to chemotherapy and hormone therapy, and it has been proven to have an effective role in Inhibiting this disease and preventing its occurrence which is one of the types of cancer diseases facing men and is widespread in many countries of the world, (Kumar *et al.*, 2016) indicated that there are about 1.7 million cases of this type of cancer in the world and the deaths of this disease reached about 499,000 thousand in various countries of the world. The research aims to prepare the mucilage of chia seeds and studying its bioactive compounds and their activities as anti-filamentary and anticancerandthese bioactive compounds could be introduced into the manufacture of medicinal drugs and used as a substitute for chemicals used in treating these diseases (Theodoropoulos *et al.*, 2016).

Materials and methods:

Detection of some bioactive compounds in the clear jelly prepared from white and black chia seeds:

Qualitative chemical detection:

The method described by (Al-zobaay and Kadhim, 2018) was used, by taking 10 grams of white and black chia seeds and adding 50 ml of distilled water to it and heating the mixture to the boiling point for 10 minutes,then let it cool down a little and separating with the centrifuge and taking the clear solution and divided into two parts. Where added 1% of lead acetate $Pb(C_2CH_3O_3)_2$ to the first part, and the presence of tannins was inferred by the appearance of a gelatinous precipitate.The second part 1% ferric chloride $Fecl_3$was added to it, whereas the appearance of the blue color indicated the presence of tannins in the mucilage.

Detection of flavonoids:

Prepare a solution (a) by dissolving 10 g of the seeds in 5 ml of ethyl alcohol at a concentration of 95% and separate the mixture with a central centrifuge at 800 rpm/minute, then the serene was taken,solution (b) was preparedby adding 10 ml of ethyl alcohol at a concentration of 50% to 10 ml of potassium hydroxide KOH at a concentration of 50%if the yellow color appears when equal quantities of solutions A and B are mixed it is indicate the presence of flavonoids in the sample(Al-zobaay and Kadhim, 2018).

Detection of glycosides:

Mix two equal parts of Fehling reagent A and B which was prepared according to the method mentioned by(Saleh and Hammadi, 2017).Solution (A): Prepared by dissolving 34 g of copper sulfate in 500 ml of distilled water And solution (B): prepared by dissolving 173 grams of Rochelle salt with 52 grams of sodium

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hydroxide in 500 ml of distilled water with the scent resulting from the aqueous extract of the seeds and let the mixture boil in a water bath at a temperature of 80 degrees for 10 minutes, and the appearance of red sediment indicated the presence of sugars represented by glycosides in the model to be examined.

Detection of alkaloids:

Boil 10 grams of white and black chia seeds with 50 ml of at a concentration of 4% and let to cool slightly and separate the mixture with the centrifuges 1000 rpm for 30 minutes, the scent wastaken then 0.5 ml of it, which represents the phytochemical in an hour bottle, was tested with the following reagents:

Meyer's reagent: The appearance of a white precipitate indicates the presence of alkaloids.

Dragendov reagent: The appearance of an orange precipitate indicates the presence of alkaloids.

Meyer's reagent solution was prepared according to the method mentioned by (Saleh and Hammadi, 2017)formed from solution (A) that was prepared by dissolving 1.35 gm of mercuric chloride in 60 ml of distilled water.And solution (B) was prepared by dissolving 5 g of potassium iodide in 10 ml of distilled water, The two solutions were mixed and made volume to 100 ml of distilled water, The Dragendov reagent was prepared by preparing two storage solutions (a) solution prepared by adding 0.6 g of Bismuth Subnitrate and 2 ml of concentrated HCl acid to (10) ml of distilled water, andsolution (B) was prepared by adding 6 g of Potassium Iodide to 10 ml of distilled water, the solution with an equal volume of (A) and (B) was mixed and add 7 ml of concentrated HCl to the mixture, then dilute the solution with it to 400 ml with distilled water.

Detection of resins:

The method mentioned in (Al-jubouri,2014)was used, add 50 ml of 95% ethyl alcohol to 5 g of white and black chia seeds and leave it to boil for two minutes in a water bath, separate the mixture with the central centrifugal 800 rpm and take the scent and add 100 ml of distilled water to it and mix well, as it is indicated for the presence of resins in the sample to be examined by the appearance of turbidity.

Detection of Coumarin:

The alcoholic extract of the white and black chia seeds was prepared by taking 10 grams of white and black chia seeds and adding 50 ml of ethyl alcohol at a concentration of 50% and placed on the electric heater at a temperature of 25°C for 30 minutes, then a quantity of it was placed in a test tube and covered with a slightly moistened filter paper with a 50% dilute NaOH solution, and the tube was placed in a water bath and left to boil for several minutes, then the filter paper was exposed to UV light source, the appearance of bright yellow-greenish color on theused filter paper it will indicate coumarin presence (Saleh and Hammadi,2017).

Quantification of some bioactive compounds in the clear mucilage prepared from white and black chia seeds:

Preparing the mucilage:

The mucilage used in the quantification of some active compounds prepared according to the method mentioned in (Dick et al., 2015), By taking 10 grams of white and black chia seeds and add 200 ml of distilled water to it, then put the mixture on a magnetic stirrer at a

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temperature of 45°C for 30 minutes, then leave it to cool slightly and distribute the mixture in test tubes and then put it in a central centrifuge (11.600 xg) for 30 minutes, and then filtered and kept in the refrigerator until use.

Tannins:

The tannins were estimated according to the method mentioned by (Aldlaly and Al-Hakim, 1989) with some modifications, as follows:

Indecocarmen index: Prepared by dissolving 1.5 g of indecocarmen in 500 ml of distilled water and heat and after complete dissolution, the solution is cooled and 50ml of sulfuric acid H_2SO_4 is added and diluted to a liter and the solution is filtered and kept until use.

Acidified table salt solution is prepared by adding 25 ml of concentrated sulfuric acid H_2SO_4 to 975 ml of saturated table salt solution.

Gelatin solution: The gelatin solution was prepared by placing 25 grams of gelatin for one hour in a saturated table salt solution and diluting the solution to a liter with distilled water.

Preparation of potassium permanganate solution: Dissolve 3.3 gm of potassium permanganate in 200 ml distilled water and transfer the contents into two-liter volumetric beakers and dilute with distilled water to the mark, Exactly weigh three samples of Na_2C_2O4 0.25-0.3 g sodium oxalate for each sample, The weighted sample is placed in a 100 ml glass beaker and 250 ml of dilute sulfuric acid H_2SO_4 is added (5 ml of sulfuric acid, 95 ml of distilled water) the contents are shaken to dissolve the model and quickly titrate with the potassium permanganate in the burette and wait until the color of the permanganate disappears and the titration is completed to the endpoint by the appearance of the pink color and the normality is calculated according to the following law:

$$Normality\ of\ permanganate = \frac{The\ weight\ of\ oxalates * 1000}{permanganate\,(ml) * 67}$$

Take 5 ml of the filtrate (prepared mucilage) and add 25 ml of indoccarmen and then dilute it to 750 ml with distilled water and titrate the contents with a solution of potassium permanganate until the color changes from blue to green and then to golden yellow and record the number of milliliters of consumed potassium permanganate, which represents the value of (A), And take 100 ml of mucilage and add 50 ml of prepared gelatin solution, then dilute the mixture to 250 ml with acidified table salt solution and take 25 ml of the prepared mixture and add 25 ml of indecocarmen, then dilute the mixture to 750 ml with distilled water and titrate the mixture with a solution of potassium permanganate until the color changed from blue to green and then to golden yellow as a point at the end of the reaction, and the number of milliliters of consumed potassium permanganate was recorded, which represents the value of (b). The amount of tannin was estimated according to the following equation:

$$Amount\ of\ tannin = \frac{(A-B) * Normality * Dulution\ factor}{Sample\ weight * 0.1\ Normality} \times 100$$

Total flavonoids:

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These compounds were estimated in Mucilage, after preparing the standard curve for the catechin compound, which was prepared by dissolving 0.05 g of catechins in 25 ml of distilled water as mentioned in (Al-Jubouri, 2014), Different volumes of catechin solution were taken and placed in test tubes and appropriate volumes of distilled water were added to them to make the final volume 2 ml. The absorbance was measured at 510 nm, as shown in Table (1).

Table (1) volumes and concentrations used in preparing the standard curve for catechins.

Number of tube	Volume of storage solution (ml)	Volume of added distilled water (ml)	Catechin concentration mg / mL
1	0.05	1.95	0.1
2	0.1	1.9	0.2
3	0.15	1.85	0.3
4	0.2	1.8	0.4
5	0.25	1.75	0.5
6	0.3	1.7	0.6
7	0.35	1.65	0.7
8	0.4	1.6	0.8
9	0.45	1.55	0.9
10	0.5	1.5	1

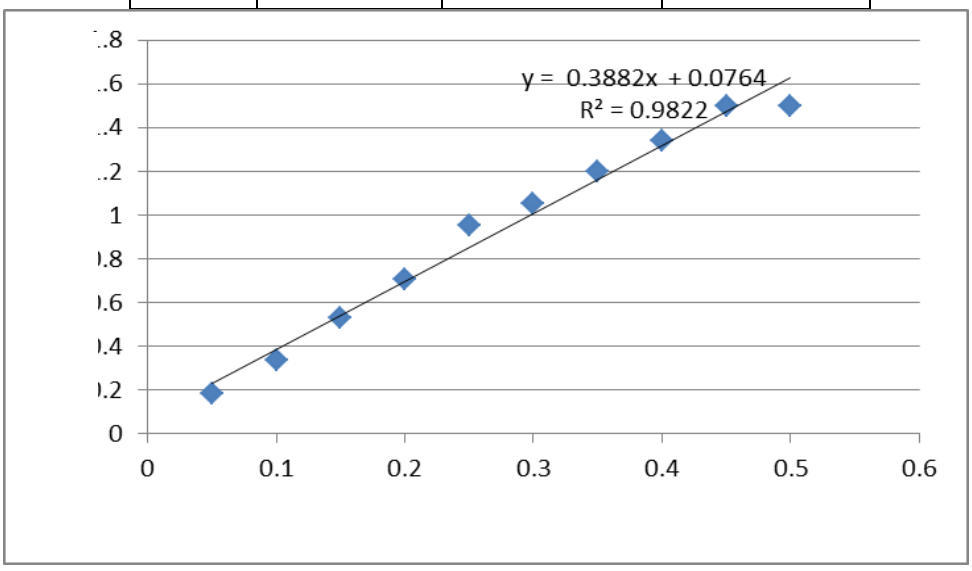

Figure (1) the standard curve of catechins

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The concentration of these compounds in the mucilage was estimated by taking 1 ml of (prepared mucilage) and placed in a 10 ml volumetric flask and 5 ml of distilled water were added, then 0.3 ml of a 5% $NaNo_2$ solution were added (prepared by dissolving 5 g of sodium nitrate in 100 ml of distilled water) after 5 minutes, 0.6 ml of 5% $AlCl_3$ solution was added (prepared by dissolving 5 ml of aluminum chloride in 100 ml of distilled water) and after another 5 minutes, 2 ml of 1 M NaOH solution were added and the volume was completed to the mark and the absorbance was measured at 510 nm and by a return to the standard curve for catechins (Figure 1)to obtain the concentration of flavonoids in the mucilage.

Estimation ofmucilage as anti-inflammatory activity:

The method mentioned by (Sandhia et al., 2015) was followed to find out the effectiveness of mucilage prepared from the two types of seeds against infections by a method called the Protein Denaturation method using bovine serum albumin 5% (W/V) solution, which was prepared by dissolving 5 grams of bovine serum In 100 ml of distilled water and a solution of Diclofenac Sodium (Aspirin) at a concentration of 250 mg/ml, the experiment was conducted with four treatments, which are as follows:

0.05 µl of Test Solution which represents the Mucilage was added to a test tube and 0.45 µl of bovine serum albumin were added, Then 0.05 µl distilled water was added in a test tube and 0.45 µl of bovine serum albumin solution were added to it and this treatment was considered the control treatment, 0.05 µl of mucilage was added to a test tube and 0.45 µl distilled water was added.Finally, 0.05 µl of Diclofenac Sodium solution was added to it and 0.45 µl of bovine serum albumin solution was added and Diclofenac Sodium solution was added as standard compound. The pH was measured for the four treatments, which should be 6.3. The reading was adjusted using (1N) HCl. The tubes were placed in a 37 m water bath for 20 minutes, and then the temperature was raised to 57 °C. The tubes were placed at that temperature for not more than 3 minutes and left to cool and added to each 2.5 ml tube of Phosphate Buffer solution with pH = 6.3 and then the absorbance was measured at 416 nm, and the inhibition ratio was calculated by using the following law:

$$Inhibition\% = \frac{100 - (O.D\ Control - O.D\ of\ Test\ Solution)}{O.D\ Control} \times 100$$

Estimation of mucilage as Anti- cancer activity:

The method described by (Mohammed et al., 2015) was adopted in estimating the efficiency of mucilage prepared from the two types of seeds as an anti-cancer against the sarcomao Rhabdomy (RD) line by using the colorimetric cell viability method. 100 µl of RD cell (610 cells/ml) was taken and distributed in 96 holes in a Tissue culture plate or by using a flat-bottomed micro culture plate and with the preparation of the test solution (mucilage) and diluted with distilled water to a concentration of 1 mg/ml. 100 µl of the extract were added to the plate containing these cells and incubated at 37°C for 24 hours, centrifugation process is carried out to expel the dead cells. After the incubation periodends, 10 µL of a solution of 5 mg/ml of 3- (4,5 dimethythiazol-2-yl) -2,5 diphenyl

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tetrazolium bromide dyeis added per hole and returned to the incubator and incubated at 37°C for 4 hours, then 50 µl of DMSO is added to each hole and incubated for 10 minutes with RD implantation in the whole medium without adding the extract (mucilage) for comparison and the absorbance was measured at 620 nm by an (ELISA)reader. And the living cells were estimated to the inhibition rate according to the following law:

$$GI\% = \frac{O.D\ control - O.D\ test}{O.D\ control} \times 100$$

Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors in study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA) or T-test was used to significantly compare between means in this study.

Results and discussion:

Table (2) shows the results of chemical tests to reveal the nature and quality of the bioactive compounds present in the clear mucilage prepared from white and black chia seeds which shown highpresence of tannins, total phenolic compounds, and flavonoids and that agree with (Kumar et al., 2016) who indicated that the mucilage contained these compounds. The mucilage contained glycosides and alkaloids, but in a smaller quantity, and did not contain both resins and coumarins as indicated by (Skrovankova et al.,2015) the mucilage prepared from these seeds contains a high percentage of flavonoids.

Table (2) Biologically active compounds in mucilage prepared from white and black chia seeds

Compound	Used indicators	Indicator index	Mucilage prepared from white seeds	Mucilage prepared from black seeds
Tannins	A-Lead Acetate 1% B-ferric chloride 1%	Form a white, gelatinous precipitate A bluish- green color appears	**++**	**++**
Flavonoids	Ethyl alcohol 95% + KOH 50%	Yellow appears	**+++**	**+++**
Glycosides	Fehling detector	The appearance of a red color precipitate	**+**	**+**
Alkaloids	Meyer's reagent Dragendov reagent	The appearance of a white color precipitate An orange color precipitate appears	**+**	**+**
Resins	Ethyl alcohol	Turbidity	**-**	**-**

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	95% + distilled water	appearance		
Coumarin	UV+NaOH	The appearance of a yellow-green color	-	-

Quantitative estimation of someactive compounds in the mucilage:

Table (3) shows the results of quantitative estimation of some active compounds present in the prepared mucilage from white and black seeds, It is noticed that the percentage of tannins present in the two types of mucilage was (35 and 38%), respectively, and the percentage of tannins that were estimated in the two types of mucilage was close to the percentage mentioned in (Vijay et al., 2014). As for the concentration of flavonoids in the two types of mucilage, it was 0.98 and 1.5 mg/ml, respectively, for the two types of seeds, and these results were consistent with what was mentioned by (Abdulkhaleq-Gazem and Chandrashekariah, 2016), (Nile and Keum, 2017) indicated that the percentage of the presence of these active compounds in the mucilage prepared from the seeds was close to the percentage of the presence of these compounds in the mucilage extracted from the leaves of some types of medicinal plants such as David's tooth plant or the sacred herb *Hyssopus officinalis* known as Zufa plant, which belongs To the *Lamiaceae* family, which is the same family as the chia plant, as well (Policegoudra et al., 2012)mentioned the importance of these compounds and their anti-inflammatory and anti-cancer role.

Table (3) quantitative estimation of some active compounds present in the mucilage prepared from white and black chia seeds

Mucilage type	Tannins%	Flavonoids (mg\ml)
White seeds	35	0.98
Black seeds	38	1.5
T-test (P-value)	2.762 * (0.0411)	0.376 * (0.0368)
* (P ≤0.05).		

The role of Mucilage extracted from both types of chia seeds (black and white) as an anti-inflammatory:

The efficiency of Mucilage, extracted from chia seeds (white and black)as an anti-inflammatory inhibitor, depends on the inhibition of protein denaturation, where protein denitrification is one of the main causes of inflammation, And through studying the effectiveness of the mucilage extracted from these seeds, as shown in Table (4) We note that the percentage of inhibition of protein denitrification by mucilage extracted from seeds was 86.5 and 97.3%, respectively, The reason for the difference in the percentage of

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inhibition between the two types of mucilage extracted from the seeds is due to the difference in the quality of the seeds between them, the method of cultivation and the type of soil, and this leads to the difference in the proportions, quantities and types of the active compounds such as phenolic compounds, flavonoids and resins, which are present in the mucilage or in the seeds themselves, which were estimated as It was mentioned previously where it is noticed that the high percentage of flavonoids in black chia seeds led to an increase in the percentage of inhibition of protein denaturation, (Reshma et al., 2014) mentioned that the percentage of inhibition of protein denitrification of the aqueous extract of the leaves of the plant *Aegle marmelos*, which is commercially known as Indian bael plants (golden, rocky, and woody apples), where the percentage of inhibition was 95.64%, and the percentage of inhibition was for phygel Higher than the percentage of inhibition of the aqueous extract of *Ocimum sanctum* leaves, which is commercially known as the holy basil plant) where the inhibition rate was 42.17%. (Sandhia et al., 2015) mentioned that the role of mucilage as an anti-inflammatory depends on the concentration of the mucilage and the relationship is positive, the higher the concentration of the mucilage, the greater its efficiency in inhibiting inflammation.

Table (4) Values of optical absorption and percentage of inhibition of mucilage extracted from both types of chia seeds (black and white)

Type of mucilage	Optical Density (416nm) Control	Optical Density (416nm) Test solution	Inhibition %
Mucilage extracted from white chia seeds	0.037	0.032	86.5
Mucilage extracted from black chia seeds		0.036	97.3
T-test (P-value)	---	0.011 NS (0.092)	4.731 * (0.0257)
* (P ≤0.05), NS: Non-Significant.			

***Each number represents an average of three replicates.**

Role of mucilage extracted from black chia seeds as anti-cancer:

The role of Mucilage extracted from chia seeds as an anti-cancer is that the mucilage contains a group of biologically active compounds that have a significant effect to be an anti-cancer, from Table (5) we noticed that the concentration of the mucilage has a positive effect in increasing the percentage of inhibition. When increasing the concentration of the mucilage to 100mg/ml, the percentage of inhibition of the tumor muscle cell line increased, so the percentage of inhibition at this concentration was 45.2%, the increase in the inhibition rate continued until at a concentration of 200mg/ml of mucilage, and the rate of inhibition was 88.4%.These results were consistent with what was mentioned by (Kumar *et al.*, 2016). The relationship is positive between the concentration of mucilage

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and the percentage of inhibition,the higher the concentration, the greater the percentage of inhibition. (Abdulkhaleq-Gazem *et al.*, 2016) mentioned that the containment of mucilage containing polysaccharides, proteins, natural catechins, flavonoids, phenolic compounds in particular, has a great role in inhibiting various types of cancers, the most important of which are colon, breast, prostate cancer in addition to the ability of these compounds on strengthening the body's immunity and limiting the growth and spread of cancer cells in the body (Women's Health, 2015).

Table (5) The role of Mucilage, extracted from black chia seeds, in inhibiting the line of muscle cell cancer

Extract concentration (mg\ml)	Inhibiting ratio GI%
50	34.8 d
100	45.2 c
150	67.8 b
200	88.4 a
LSD value	6.935 *
This means having the different letters in the same column differed significantly. * (P ≤0.05).	

***Each number represents the average of two replicates**

Conclusion:

It is clear that mucilage has many functional activities for its content of tannins, glycosides, flavonoids, phenols which act as anti-filamentary and anticancer. These properties encourage researchers to make further studies on animals to find perfect dosages of using chia seeds in cancer treatments.

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