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IN-VITRO STUDY OF ANTI-DIABETIC, ANTI-CANCER AND ANTI-UROLITHIATIC ACTIVITY OF DIFFERENT EXTRACTS OF *PROSOPIS JULIFLORA* SEEDS

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ABSTRACT: Plants and their parts are used to treat many infectious diseases due to the presence of phytoconstituents and avoid some side effects. The present study evaluated the phytochemicals, anti-diabetic, anti-urolithiatic and anti-cancer activities of *Prosopis juliflora* seeds in various solvents such as aqueous, ethanol and 85% ethanol. The preliminary works revealed the presence of secondary metabolites likes alkaloid, phenol, saponin, tannin, phytosterol and flavonoids. The work focused on the anti-diabetic properties of the extract by α-amylase, α-glucosidase methods. *In-vitro* anti-urolithiatic activity by titrimetric method showed that there was maximum activity, and its effect was compared with the standard drug cystone. The MTT assay was used to screen the crude extracts and the isolated compounds to assess their toxicity. The aqueous extracts of *Prosopis juliflora* seeds showed the inhibition effects of the cancer cell lines (MCF7), while there was no effect on the growth of normal cells. Such selective effects were found to be time-dependent. With respect to concentration (25 µg/ml, 50 µg /ml, 100 µg /ml, 250 µg /ml and 500 µg /ml,) of extract were evaluated in triplicates by serial dilution. Among these, 500 µg /ml of ethanol extract was the most effective in producing percentage growth inhibition. The results showed that the aqueous extract significantly inhibited the (MCF7) cancer cell lines. So, seeds of *Prosopis juliflora* extract can be exploding further to produce active herbal principles for therapeutic use.

INTRODUCTION: All over the world has covered with water and land which have more specified medicinal values. Herbal plants possess certain medicinal properties. Nowadays, herbal products have become an important matter in industrialized areas and in developing countries declaration of the protection, effects, and values of the medicinal plants. In our ancient period, herbal plants were used for supplementary diet and to cure disorders.

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Most of the plants have tremendous medicinal properties to treated, prevented ailments [1]. Plants always synthesis the stress-protective shield against the photosynthetic stress caused by the exogenous exposure. Plants provide primary and secondary metabolites and it considers to be a bioactive component. It is used for industrial purposes to make the drug, medicine, antibiotics, *etc.*, the normal bioactive substances present in the leaves, flowers, seeds and other branches of plants. The plants also protect against the infection-causing microorganisms presented in the soil.

Plants used as a natural fertilizer for good yielding. A dietary source rich in anti-oxidant properties has been chosen in this study. They have been known to exhibit potential health-beneficial properties against various disorders in traditional medicine.

Prosopis juliflora: *Prosopis juliflora* is an exotic species and woody species, grown in tropical areas highly evergreen plant and mostly it appears as a shrub and thorny trees its grows up to 12-14 feet, stem brown-green, twisted flowers side to the axis of the stalk and light yellow color when mature flower, thorny branches, 2-3 cm thick barks and the flowering seasons of pods August-September, November- December, February- March [2].

A pod regularly appears flat, straight, 5-30 cm long, 6-10 mm wide, 3-8 mm thickness. Pods are looked fluffy, pulpy, and yellowish-greenish color. Seeds are shiny, flatten, hard and yellowish-brown color. The distribution of *Prosopis juliflora* worldwide can grow in all the soil varieties like coastal areas, saline areas, grasslands and dunes areas and dry areas [3]. *Prosopis juliflora* consists of extractives, interactive composition and interactive chemicals: ash, water, cellulose, lignin and hemicellulose.

Extractives chemicals of sugar, volatile oil, resin, fatty acids, and other secondary metabolites and chemical component enrichment improve the property of the plants. The sugar component of mannose and galactose shows the presents of carbohydrates. Leaves of the *Prosopis juliflora* supply the excessive resource of antioxidant capability and prevent cardiovascular diseases and cancer due to flavonoids and other secondary metabolites. Phenols are reported to prevent the platelets from clumping and have the ability to block the specific enzymes that cause inflammation. These also act as immune enhancers, anti-clotting, and hormone modulators. Tannins in the plant cell inhibit hydrolytic enzymes like proteolytic macerating enzymes used by plant pathogens [4]; the tree has been regarded as a valuable resource for firewood and dune stabilization [5]. Seeds are a potential source of seed gum. Free-ranging animals can eat pods directly from the tree. Alternatively, the pods can be collected and grounded to produce coarse flour, which can be included in the animal's diet, and the flour has anti-mutagenic effects. Pods galactomannan has good effect used in the food industry and physicochemical properties [6]. Parts of this plant are used for certain therapeutic effects like Itching, skin lesions, Measles, Stomach ache, Sore throat, Hoarseness, excrescences, Flu, digestive disorder, dysentery, diarrhea, catarrh, cold, and it's a best for disinfectant. *P. juliflora* syrup prepared from ground pods is given to children showing weight deficiency or retardation in motor development; the syrup is believed to increase lactation [7]. Tea made from *P. juliflora* is thought to be good for digestive disturbances and skin lesions. It has soothing, astringent, antiseptic, antibacterial and antifungal properties [8]. It has been used to treat eye problems, open wounds, dermatological ailments and digestive problems by the native tribes of many countries. The flavonoid patulitrin isolated from its flowers and fruits showed significant activity against lung carcinoma *in-vivo* [9]. Present scenario to investigate the phytochemicals, anti-diabetic, anti-urolithiatic and anti-cancer activities of *Prosopis juliflora* seeds in various solvents such as aqueous, ethanol and 85% ethanol.

MATERIALS AND METHODS:

Preparation of Extracts by Soxhlet Method: *Prosopis juliflora* seeds were collected from the areas in Puducherry in March, (2018). Seeds were dried under shade for 2 weeks, crushed into powder by using an electronic blender, and sieved to 360 mesh pore size. Successive different solvents were used to prepare the extracts by using the Soxhlet extraction method. Twenty grams of dry powder use to extract preparation with 200 ml of Aqueous (distilled water), ethanol, and 85% ethanol were used in Soxhlet. After extraction, they were transferred to rotor evaporator to decrease the pressure and dried for 40 °C at 30 min [10].

Test for Phytochemicals: The various tests were carried out to screen the active constituents in three extracts: Aqueous (distilled water), ethanol and 85% ethanol.

Anti-diabetic Activity:

α-glucosidase Inhibition Assay: The Alpha-glucosidase inhibitory activities were performed with sets of testing tubes which were mentioned as blank, controls and samples (different concentrations) and were set up in the stand. The reaction mixture contains 150 µl of 0.1 M PBS (pH 7.4) 75 µl of 20 mm PNPG solution and added 25 µl of the sample were added after that the mixture was incubated at 37 °C for 10 min. Then the reaction was initiated by adding 1mL of 0.1 M

Na_2CO_3. The amount of p-nitro phenol released was determined by estimating the absorbance at 405 nm. For blank, the reagent was added in a reverse manner. A set of color control was done for the test samples without enzyme and PNPG but maintaining the buffer volume.

The absorbance of test tubes was noted at 405 nm using UV. The percentage of inhibition of enzyme action by test samples was calculated [11, 12] and incubated for 40 min at 35 °C followed to the initial reaction and ad 2 ml of HCL to terminate the reaction.

α-Amylase Inhibition Assay: 500 µL of test compound was added to the standard antibiotic (100-500 µg/ml). The 500 µl of solution contain 0.20 m MPBS buffer (pH 7.4) to α-amylase (0.5 mg/ml) solution and incubated at 25 °C for 10 min. 1% of starch solution and 0.02 M sodium phosphate buffer (pH 7.4) were added to all the test tubes.

Again it was incubated with similar at 25 °C. Then 1 ml of 3,5 di-nitro salicylic acid was added to stop the reaction and placed in a water bath for 5 min, placed at room temperature. Then reaction absorbance was recorded and measured at 540 nm [13, 14].

Antiproliferative Activity: The Breast cancer cell line (MCF7) were plated separately using 96 well plates with the concentration of 1 × 104 cells/well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO_2 incubator at 37 ºC with 5% CO_2.

The cells were washed with 200 µl of 1X PBS and then the cells were treated with various test concentrations of the compound in serum-free media and incubated for 24 h.

The medium was aspirated from cells at the end of the treatment period. 0.5mg/ml of MTT prepared in 1X PBS and incubated at 37 ºC for 4 h using CO_2 incubator. After the incubation period, the medium containing MTT was discarded from the cells and washed using 200 µl of PBS. The formed crystals were dissolved with 100 µl of DMSO and mixed thoroughly. The development of color intensity was read at 570 nm.

The formazan dye turned to purple-blue color; the absorbance was measured at 570 nm using microplate reader [15, 16].

Anti Urolithiatic Activity:

Calcium Oxalate Preparation: Kidney stones (calcium oxalate) synthesized by artificial homogenate precipitation protocol. Equimolar solution of water dissolved calcium chloride dihydrate and 2N sulfuric acid added sodium oxalate were mixed then allowed to stand for few hours.

The calcium oxalate precipitates were formed. The formed precipitates were collected and washed with excess ammonia solution to free from traces of H_2SO_4. After that, the precipitate was washed again with distilled water. Finally, the dried calcium oxalate was used as an artificial stone for this experiment [17].

FIG. 1 & 2: PRECIPITATION OF CALCIUM OXALATE

Egg Membrane Preparation: The semi-permeable membrane preparation was done by Chicken eggs for this experiment. The eggs were soaked in 2 M HCl solution at 12 h for

decalcification then the inner content was squeezed out carefully by puncturing a hole at the top of the decalcified egg. Then the membrane sac was washed with ammonia solution then washed with distilled water. The prepared membrane sacs were stored at 4 °C for further use.


FIG. 3 & 4: EGGS ARE SOAKED IN 2M HCL


FIG. 5 & 6: PREPARED MEMBRANE SAC

Estimation of Calcium Oxalate by Titrimetric Method: About 1mg of the calcium oxalate and 10, 20, 30 mg/ml of the extracts were packed in the semi-permeable membrane. The membrane was sutured at one end and placed in a conical flask containing 100 ml of 0.1 M Tris buffer. Calcium oxalate without the extract serves as a negative control. Cystone was used as a positive control. The conical flasks were incubated in a pre-incubated chamber at 37 ºC for about 8 h. Following incubation, the contents from the sac were collected into a test tube. To the contents, 2 ml of 1N sulphuric acid was added and titrated against the 0.94N $KMnO_4$ till the appearance of light pink color. 1 ml of 0.9N $KMnO_4$ is equivalent to 0.1898 mg of calcium. The percent dissolution of the calcium oxalate was calculated using the amount of undissolved calcium oxalate [18].

RESULTS:

Phytochemical Screening:

TABLE 1: QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN *PROSOPIS JULIFLORA* SEED EXTRACTS

S. no.	Phyto Constituent	Tests	Aqueous Extract	Ethanolic Extract	85% Ethanolic Extract
1	Carbohydrates & Glycosides	Molisch's	+++	+	++
		Fehling's	+++	+	++
		Benedict's	+++	+	++
		Barford's	+++	+	++
2	Alkaloids	Mayer's	+++	+	++
		Wagner's	+++	+	++
3	Saponins	Foam test	++	+	+
		Froth test	++	_	+

4	Protein & Amino acids	Biuret	+	+	+
		Ninhydrin	–	–	–
		Xanthoproteic	+	+	+
5	Phenolic compounds	Ferric chloride	++	–	+ +
6	Flavonoids	conc H_2SO_4	+++	++	++
7	Phytosterol	Libermannburchard	+++	+	++
8	Tannins	Gelatin test	+++	++	++
9	Diterpenes	Copper acetate test	++	+	+

Anti Diabetic Activity:

Anti Diabetic activity of α-glucosidase and α-amylase Method:

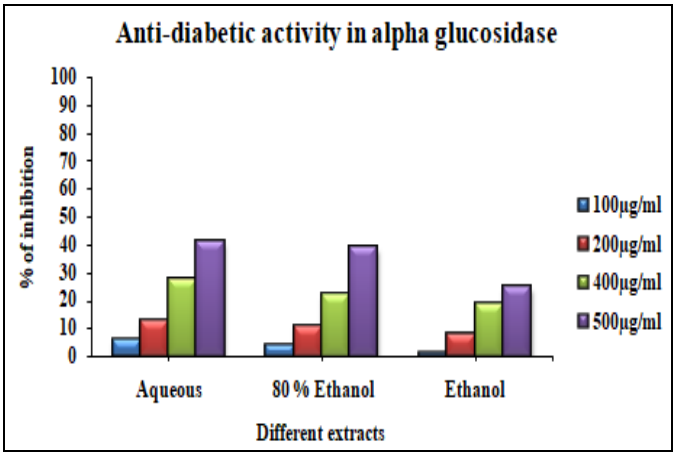


FIG. 7: EFFECT OF *PROSOPIS JULIFLORA* ON ANTI-DIABETIC ACTIVITY

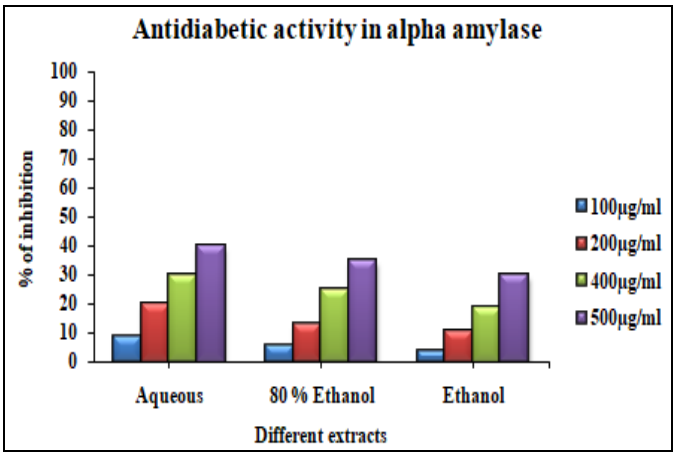
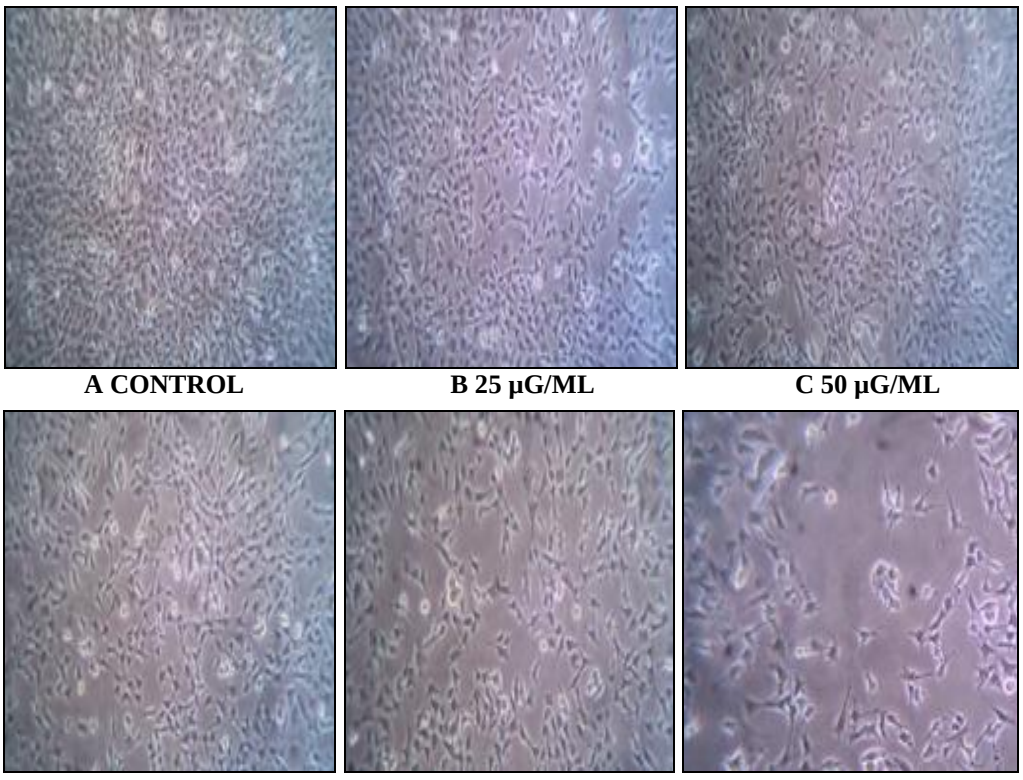


FIG. 8: EFFECT OF *PROSOPIS JULIFLORA* ON ANTI-DIABETIC ACTIVITY

Anticancer Activity:



A CONTROL **B 25 µG/ML** **C 50 µG/ML**

D 100 µG/ML **E 250 µG/ML** **F 500 µG/ML**

FIG. 9: *IN-VITRO* MEASUREMENT OF CELL VIABILITY OF AQUEOUS EXTRACT OF *PROSOPIS JULIFLORA* SEEDS

Anti-urolithiatic Activity:

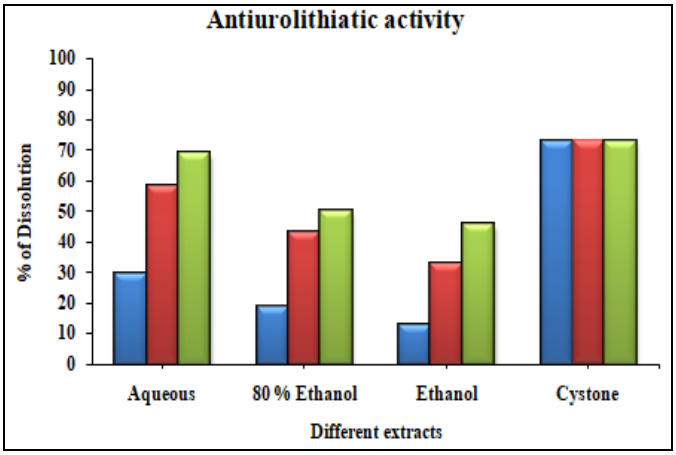


FIG. 10: EFFECT OF *PROSOPIS JULIFLORA* SEEDS EXTRACT ON CALCIUM OXALATE

DISCUSSION: The main functions of secondary metabolites are efficient in fighting against various diseases and give an aromatic smell. The phytochemical analysis on various extracts of *Prosopis juliflora* (aqueous, ethanol, 85% ethanol) showed that phytoconstituents like carbohydrates, phenols, alkaloids, flavonoids, tannins, saponins, and diterpenes, respectively. The results were tabulated in **Table 1.** These act as good antioxidants, mainly scavenge the free radicals.

Alkaloids are pharmaceutically significant and are used as an analgesic, antimalarial, antiarrhythmic, antispasmodic, in treating cough and pain, gout treatment, and pupil dilatin [19]. Polyphenol content act as good electron donors and certain plant Phenolic compounds acts on α-amylase and inhibit the activity so the phenolic compounds are useful in the diabetic patient (Type-2). Flavonoids involved in many biological events including free radical scavenging property and it possesses strong and good anti-inflammatory properties [20].

DM is the third leading cause of death in the world. It is a metabolic disorder characterized by high blood glucose resulting from defects in insulin secretion. The most common type of diabetes is type-2diabetes which is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Chronic hyperglycemia may induce oxidative stress that may lead to long-term damage, dysfunction, and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels [21].

The natural sources respect to suppression of glucose production from the carbohydrates in the gut or glucose absorption from the intestine. α -amylase catalyzed the hydrolysis of α-1,4-glycosidic linkage of starch, glycogen, and various oligosaccharides [22]. The α-glucosidase further breakdown the disaccharides into simple sugars, readily available for intestinal absorption. The inhibition of their activity in the digestive tract of humans is considered to be an effective tool to control diabetes. In addition, these effects may lead to diminished absorption of monosaccharide [23].

Therefore, effective and nontoxic inhibitors of alpha-amylase and alpha-glucosidase have long been sought [24, 25]. α-glucosidase enzymes in *Prosopis juliflora* seed extract shows the concentration of 100, 200, 400, 500 µg/ml and analysis the glucose uptake can be founded after cells were treated with the test sample and minimize the uptake of glucose from the intestine, adipose tissue leads to increase the blood glucose condition. Then the three extracts exhibit potential antidiabetic activity effect in dose-dependent mode. Most significantly, aqueous extract exhibits a high range of % of dissolution compared with 85% ethanol, ethanol extracts. In the α-amylase activity of various extracts have high % of inhibition of α-amylase enzymes in *Prosopis juliflora* seed extracts exhibits at concentration of 100, 200, 400, 500 µg/ml and analysis the uptake of pancreatic amylase can be founded after cells were treated with test sample and test compound slow down the absorption and digestive form of starch and carbohydrates.

Then the extracts revealed potential antidiabetic activity effect in a dose-dependent mode. The aqueous extract has a high % dissolution compared with 85% ethanol, ethanol extracts. The antioxidant activity has been known as a considerable resource of plant drugs allied with diabetes. Herbal medicine has been used in developing countries as the primary source of cancer treatment. Generally, the plants have natural antiseptic activities. Thus, more research has developed into investigating the potential properties and uses of terrestrial plant extracts to prepare potential nonmaterial-based drugs for diseases, including cancer. The World Health Organization announced the plant-based treatments are the best source for medicine and

therapeutic purposes. The biologically important compounds include polyphenol, brassinosteroids, and taxol from terrestrial plants for cancer treatments [26]. Anticancer activity shows in Prosopis juliflora seed extracts exhibit in % inhibition in the cell viability range. MTT assay basis for declining the cytotoxic level by MTT product purple colour formazan. This procedure was followed to eradicate the in vitro human cell line to measure the cytotoxic effect caused by carcinogenic substances by using MCF 7 (breast cancer). The *Prosopis juliflora* seed aqueous extract showed that reduce the in vitro growth of cancerous cells and with concentration 25 µgml, 50 µg/ml, 100 µg/ml and 250 µg/ml, 500 µg/ml were used. The result of aqueous solvent effectively involved in cancerous cell (MCF 7) to restrict significantly. Renal calculi, a common multi-fractional disorder, affects all age groups of people. The stone formation takes place in the renal system known as urolithiasis. In the world, the top most diseases affected the people due to some renal disease. In anti urolithiatic activity of the seed, extracts show the beneficial medicinal effect at the conc. of 100, 200, and 400 µg/ml expressed the % of dissolution.

The dissolution of calcium oxalate stone by titrimetric method with different extracts inhibited the calculi compared to the standard antibiotic drug Cystone (100 µg/ml). In aqueous extracts showed the increase at 400 µg/ml (68.92), 85% ethanol showed the increase at 400µg/ml (50.02), and ethanol extract showed the increase at 400 µg/ml (45.72). The aqueous solvent shows a more significant effect compared to 85% ethanol and ethanol extracts. The current medical system has not been satisfied due to the lacking of clinically used drugs that can be used to dissolve the renal stones or to prevent stone formation. So that the herbal remedies only provided better results for stone treatment. Several studies confirmed the beneficial properties of these phytochemicals in the prevention and treatment of urolithiasis. Recently Noorafshan reported that flavonoids inhibit CaOx crystallization in human urine and in animal models [27]. Similarly, saponins possess an anti-crystallization property by disaggregating the suspension of mucoprotein as the promoters of crystallization reaction [28]. Thus, we conclude that the antiurolithiatic activity observed in the present study was due to the synergetic effect of these phytochemicals present in the extracts of *Prosopis juliflora* seeds.

CONCLUSION: In anti-diabetic activity showed aqueous extract has significant effects in both enzymes (α-glucosidase and α-amylase inhibitory) in other extracts at the beneficial concentration. These activities involved reducing the sugar level in digestion and absorption. The plant extract has the ability to decrease blood glucose levels. In anti-cancer action revealed the potential effects in aqueous extracts has tumor cell proliferation and study the cytotoxic inhibitory properties.

MCF 7 cell line 250 µg/ml concentrations to elevate the cell death and cell proliferation mechanism were observed. Anti-urolithiatic activity showed the anti lithiasis action. This study showed the aqueous extract express a more significant effect at the concentration 400 µg/ml increased dissolution percentage the calculus formation compared to the 85% ethanol and ethanol extracts in pharmacological and *in-vitro* studies.

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CONFLICTS OS INTEREST: None

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