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Research Article

Integrative Evaluation of Harpagophytum procumbens Root Extract for Anticancer Activity: A Network Pharmacology and In Vitro Approach

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Abstract

This study explores the anticancer efficacy of the ethanolic extract of Harpagophytum procumbens by integrating network pharmacology analysis with in vitro evaluation on prostate cancer cell lines. Preliminary phytochemical screening confirmed the presence of several bioactive groups such as phenols, flavonoids, steroids, saponins, alkaloids, tannins, and glycosides. Through network pharmacology, rutin, harpagoside, stigmasterol, and β-sitosterol were identified as the major active constituents, all demonstrating favorable drug-likeness properties. ADMET analysis, molecular target prediction, and protein–protein interaction (PPI) network mapping revealed vital cancer-associated targets including TP53, AKT1, VEGFA, EGFR, and MAPK1. Functional enrichment analyses (GO and KEGG) indicated that these compounds influence several critical pathways related to cancer progression, especially those governing apoptosis, cell cycle regulation, and MAPK and PI3K–Akt signaling cascades. Cytoscape-based interaction mapping revealed that rutin interacts with several targets, including MAP2K1, AKT1, MMP2, SRC, and MAPK1, indicating the multi-target therapeutic potential of the extract. Additionally, the MTT assay results showed that the ethanolic extract exhibited dose-dependent cytotoxicity against prostate cancer cells, with an effect comparable to the standard chemotherapeutic drug 5-fluorouracil.

Keywords: Prostate Cancer, Network Pharmacology, Anticancer Activity, MTT assay.

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Introduction

Cancer is defined as the lack of normal regulatory mechanisms that govern cell survival, proliferation, and differentiation.(1) Prostate cancer originates in the prostate, a small, walnut-sized gland in men that plays a role in producing seminal fluid, which nourishes and transports sperm.(2) A number of factors influence the incidence, geographic distribution, and behavior of specific forms of cancer, including age, gender, race, nutrition (high calcium consumption), ionizing radiation genetic predisposition, and pollution exposure. Prostate cancer is common among males and ranks as the fifth most common cause of mortality. Prostate cancer is diagnosed by PSA, digital rectal examination, and transrectal ultrasonography (TRUS).

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The main medical treatment for prostate cancer is antiandrogen therapy; examples include fluatamide, betalutamide, nitamide, leuprolide, dutasteride, goserelin, histrelin, and triptorelin
Prostate cancer makes for 15% of all cancers and is the most frequent malignancy in men in 112 nations. Prostate cancer case estimates for 2040 are based on statistics from worldwide population changes and rising life expectancy. It is anticipated that the annual incidence of new cases will increase from 1.4 million in 2020 to nearly 2.9 million by the year. According to the aforementioned, the estimated increased prevalence of prostate cancer in 2040 will gradually increase. (3,4)

Due to a lack of awareness and limited PSA testing, the majority of cases are diagnosed at the metastatic stage, which leads to significant fatality rates. The incidence rate of prostate cancer adjusted for age is 4.8 incidences per lakh people annually, with a significant increase of 30% nationally and 75-80% in metropolitan regions over the last 25 years. In order to treat the disease's burden in India, it is essential to comprehend the one national cancer database. Therefore, this issue pertains to prostate malignancies and is the focus of our research topic, with a focus on natural pharmaceutical therapy.(5)

Network Pharmacology and *in vitro* methods are used to learn more about the molecular mechanisms and therapeutic potential of these compounds in the plant Harpagophytum procumbens.(6) Because of their effectiveness and affordability, we prefer *in vitro* techniques over *in vivo* ones in our investigation. Before advancing to more intricate *in vivo* models, in vitro research offers important early insights and enables quick testing of anticancer drugs in a controlled setting.(7)

Devil's claw, or *Harpagophytum procumbens*, is a medicinal plant that is a member of the Pedaliaceae family. Its roots, seeds, fruits, and leaves have been tested for a variety of medicinal purposes, including the treatment of rheumatism, dyspepsia, urinary tract infections, sprains, gastric ulcers, menstrual cramps, arthritis, and anti-mutagenicity. Flavonoids, Phenolic Compounds, Harpagoquinones, Amino Acids, Phytosterols, Carbohydrates, and Phenylethanoid Glycosides are the seven phytoconstituents found in Harpagophytum procumbens.(8,9) There are, however, insufficient scientific investigations on the antioxidant and antiangiogenic effects of H. procumbens. (6) The persistence of this study is to explore H. procumbens extract's anticancer properties. (7)

Materials and Methods

Test Drug

Test drug sample received as a gift sample of authenticated dry root extract of *Harpagophytum procumbens*(100gm) from HHC.AXICOMS Private limited, Belgaum-590015.

Harpagophytum procumbens bioactives identification and target screening

Using the keyword *Harpagophytum procumbens*, Dr. Duke's (http://phytochem.nal.usda.gov/) collection of phytochemical and ethnobotanical information and scientific journals were searched for the bioactive chemical contents of *Harpagophytum procumbens*. The Chemical compound database (10)(https://pubchem.ncbi.nlm.nih.gov/) was employed to obtain Compound Identification Numbers (CIDs) and Canonical SMILES representations for chemical compounds associated with specific protein targets.(11) Canonical SMILES were then enquired for target prediction in Binding DB(12) with a probability score of 0.7 (70%). Uni Prot provided the gene ID for each protein. The protein molecules associated with prostate cancer were further searched based on the approved and successful targets listed in the Therapeutic Target Database (TTD). (13)

Drug likeness and possible side effects of *Harpagophytum procumbens* chemical constituents

The drug-likeness of the chemical constituents was assessed using Molsoft (https://molsoft.com/mprop/). (14,15) This online tool evaluates the potential of a compound to exhibit drug-like properties. In this analysis, Compounds were screened using Lipinski's Rule of Five to exclude those that did not meet essential drug-likeness criteria—namely, having over 10 hydrogen bond acceptors, more than 5 hydrogen bond donors, a molecular weight above 500 g/mol, or a log P value exceeding 5. To predict possible adverse effects of the compounds, the ADVERPred online tool (https://www.way2drug.com/adverpred/) was utilized. (16) This tool leverages structure–activity relationship (SAR) data and information on common and serious drug-related adverse reactions to forecast the toxicity profile of a chemical. This study utilized ADVERPred to assess the possible adversative effects of the selected chemical composites. The tool was used to calculate the probability of activity (Pa) and inactivity (Pi) for each compound.This experiment was conducted using the admetSAR2 (http://lmmd.ecust.edu.cn/admetsar2/)(17)About 2 million wet lab data points for 0.96 lakh drug candidates are included in this online platform. comprises 27 computer models that can be used to predict ADMET profiles, including mutagenicity, oral bioavailability, plasma protein binding affinity, BBB permeability. (18,19)

Network formation and gene enrichment analysis

The identified protein targets were analyzed for interactions using the STRING database (https://stringdb.org). To explore their roles in cancer-associated biological pathways, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/pathway.html) was employed. Subsequently, Cytoscape v3.7.2 was used to visualize and construct a net linking compounds, targets, and paths. This network was further analyzed to identify the key compounds, targets, and signaling pathways involved in the progression of prostate cancer.

In vitro Method

MTT Assay

PC3 prostate cancer cells were cultivated at a density of 1×10^4 cells/ml in DMEM with high glucose (Dulbeccos Modified Eagle Medium) growth media and incubated for 24 hours at 37°C in a humidified environment with 5% CO_2. Then, 10^4 cells per well were planted into 96-well tissue culture plates with 100 µl of DMEM. The H. procumbens extract samples were introduced to the wells at varying concentrations (25, 50, 100, 150, and 200 µg/ml). The control wells received only 0.2% DMSO in PBS, along with the PC3 (Prostate Cancer cell line 3). All treatments, including the control, were carried out in triplicate. The cultures were maintained for 24 hours under normal conditions (37°C, 5% CO_2). After the incubation period, thoroughly aspirate the medium and 20 µl of MTT reagent (5 mg/ml in PBS) was added to each well. Following the addition of MTT, the cells were further processed to determine viability. After adding MTT, cells were cultured in a CO^2 incubator for 4 hours at 37°C. The wells were examined under a microscope for formazan crystal development. The absorbance of the dissolved formazan crystals was measured at 570 nm using a microplate reader after the addition of 200 µl of DMSO. Only living cells could convert yellowish MTT to dark-colored formazan. After removing the medium completely. Incubate at 370C for 10 minutes after adding 200µl of DMSO and wrapping in aluminum foil. (20,21) The percentage inhibition and IC_{50} value can be calculated using the following formula:

$$\% \text{ Inhibition} = ((\text{Control response} - \text{Inhibitory response}) / \text{Control response}) \times 100$$

Where,
Control response = Absorbance of untreated control cells
Test/Inhibitory response = Absorbance of cells treated with the test sample

Results and Discussion

Chemical Constituents and their targets

Harpagophytum procumbens was reported to have potential anticancer activity. which may due to aquistation of flavonoids, polyphenols, steroids as major secondary metabolites by them. The numerous chemical ingredients found in Harpagophytum procumbens were extracted using literature support.(11) Around 42 possible active ingredients were identified from these literatures, the majority of which are flavonoids, polyphenols, and steroids.

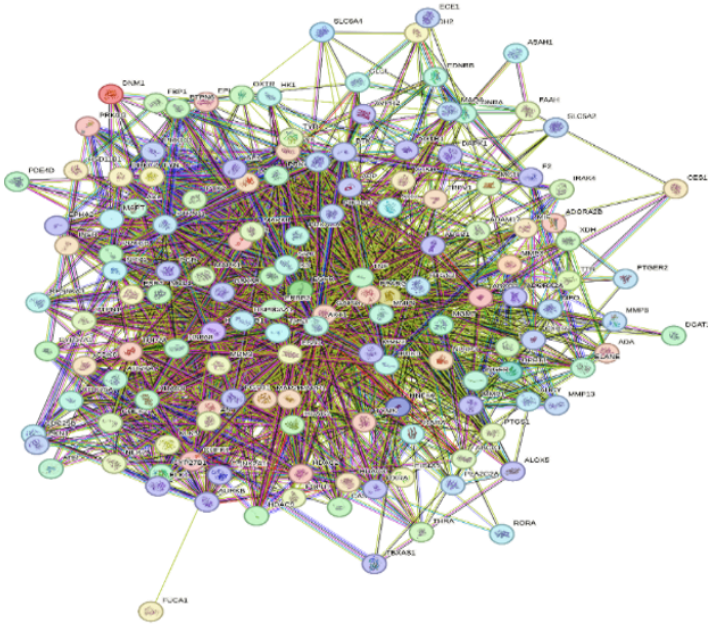
Compounds with a drug similarity score of ≥1 are further evaluated for their potential function in prostate cancer. For each 14 screened chemical entities different protein targets were retrieved with help of Swiss Target Prediction .there were total 491 targets of phytocompound 14 were retrived and 457 total disease targets were retrived from genecard database by selecting prostate cancer,(Table 1) and then common targets of both phytocompound and disease related targets were retrived by using VLOOKUP formula(=VLOOKUP(D94,$F$1:$F$5000,1,FALSE) and total 170 targets were from that and that further use for gene analysis enrichment pathway and Protein-protein interaction of this retrieved protein was constructed with help of STRING database (Figure1).

Table 1: Active ingredients and conformity to the drug-likeness criteria according to Lipinski's rule of five

Bioactives	Molecular formula	Molecular weight	NHBA[a]	NHBD[b]	Mol LogP[c]	MolLogS[d]		MolPSA (A2)	Mol Vol (A3)	DLS[e]
						Log	mg/l			
3-beta-acetyl-oleanolic acid	C32H50O4	498.37	4	1	7.21	-5.66	1.08	49.69	637.62	0.38
Acetoside	C29H36 O15	624.21	15	9	-0.15	-1.44	22890.62	196.80	556.39	0.51
Verbascoside	C28H34O15	610.19	15	9	-0.96	-1.43	22743.38	199.45	555.29	0.69
Beta-carotene	C40H56	536.44	0	0	13.93	-6.08	0.45	0.00	829.01	0.64
Chlorogenic-acid	C16H18O9	354.10	9	6	-0.20	-1.40	14035.24	127.41	338.61	0.79
Harpagide	C15H15NO3	257.11	3	1	2.68	-2.84	375.19	43.86	262.08	0.29
Kaempferol	C15H15NO3	257.11	3	1	2.68	-2.84	375.19	43.86	262.08	0.29
Quercetin	C15H10O7	302.04	7	5	1.19	-2.19	1952.89	102.61	281.71	0.52
Luteolin	C15H10O6	286.05	6	4	2.78	-3.11	224.49	89.05	272.86	0.38
Rutin	C27H30O16	610.15	16	10	-1.55	-1.75	10775.79	213.63	533.42	0.91
B-Sitosterol	C29H50O	414.39	1	1	8.45	-6.34	0.19	16.28	519.36	0.78
Stigmasterol	C29H48O	412.37	1	1	7.74	-6.24	0.24	16.28	529.89	0.62
Ursolic acid	C30H48O3	456.36	3	2	6.46	-5.60	1.15	44.14	576.10	0.66
Oleanolic acid	C30H48O3	456.36	3	2	6.66	-5.63	1.07	44.14	593.27	0.37

a) NHBA: Count of Hydrogen Bond Acceptors; b) NHBD: Count of Hydrogen Bond Donors; c) MolLogP: Logarithmic value of the partition coefficient, indicating lipophilicity; d) MolLogS: Logarithmic value representing aqueous solubility; e) DLS: Drug-likeness score, reflecting the compound's potential as a drug candidate

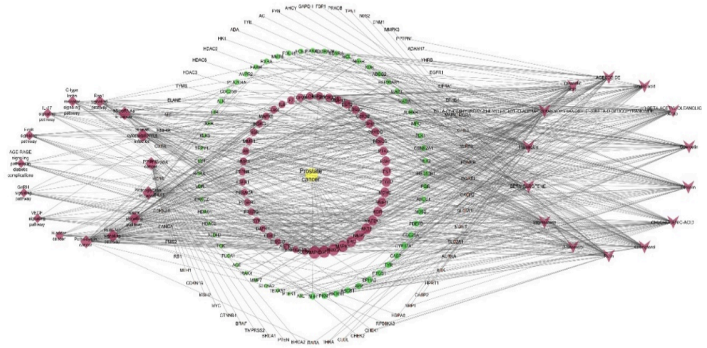
Figure 1: Protein-protein interaction of target retrieved by STRING Database.



Network construction and Analysis

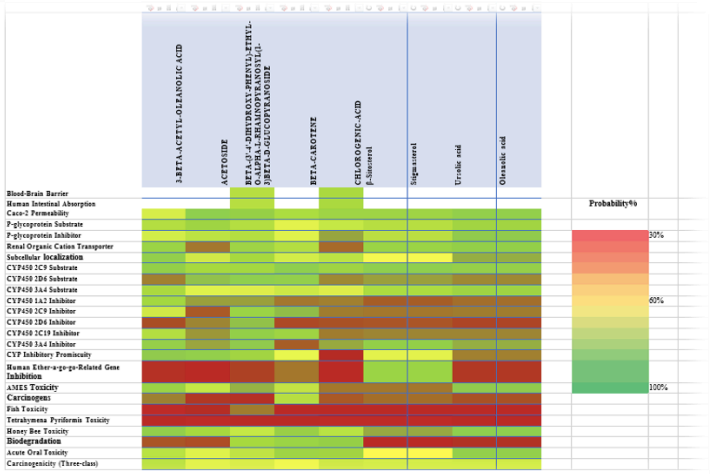
Network construction between Phytoconstituents, Pathway and Disease Targets. An analysis of KEGG pathways revealed 15 pathways intricately linked to the pathogenesis of prostate cancer with the IL-17 signalling pathway demonstrating the highest number sets of gene at a false discovery rate of 7.23E-23. Notably, Rutin, a phytoconstituent of *Harpagophytum procumbens* was found to interact with five protein molecules within this pathway including. MAP 2K1, AKT1, MMP2, SRC and MAPK1. Furthermore, a network pharmacology-based predictive analysis of *Harpagophytum procumbens* constituents identified key pathways implicated in prostate cancer, including the PI3K-AKT pathway, MAPK signalling pathway, Ras signalling pathway, there by inhibiting their growth (Figure 2).

Figure 2: Network indicating interaction of Phytoconstituents, Pathway and Disease Targets



Adsorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET):

Figure 3: ADMET profile of screened compounds in heat map representation



The ADMET profile for all nine chemical constituents was anticipated and the drug-likeness score for all nine chemical constituents was positive. The greatest drug-likeness score was 0.78 for β-Sitosterol. All of the chemical constituents potential adverse effects were predicted. 3beta-acetyl-oleanolicacid, acetoside, verbacoside, chlorogenic-acid, β-sitosterol, stigmasterol, ursolic acid, oleanolic acid, beta-carotenewere predicted to be hepatotoxic, nephrotoxic, myocardial infarction with their probability. The absorptivity of bioactive chemical constituents, its acute toxicity, BBB permeability, PPB, mutagenicity, isoenzyme inhibitory activity, carcinogenicity and other ADMET profiles were anticipated, and a heat map with a percentile of 30–100% represents the overall ADMET profile (Figure 3).

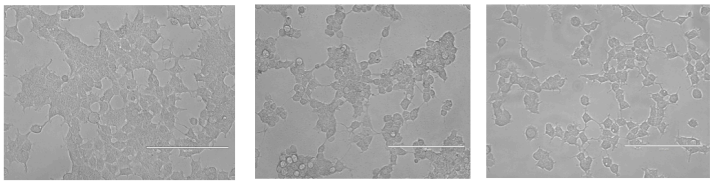
In vitro Cytotoxic Activity

The MTT assay results demonstrated that the ethanolic extract exhibited significant dose-dependent cytotoxic activity against PC3 prostate cancer cells (Table 2). The observed inhibitory effect increased with concentration, indicating the potential anticancer activity of the extract. 5-Fluorouracil was selected as the standard drug because it is a well-established chemotherapeutic agent widely used in cancer research and acts by inhibiting DNA synthesis in rapidly proliferating cancer cells, making it an appropriate reference for evaluating cytotoxic efficacy. The comparable inhibitory response of the extract with the standard drug suggests its promising potential as a multi-target anticancer candidate.

Table 2: The effect of *Harpagophytum procumbens* root extract of MTT assay on PC3

SR NO	Sample	Conc. (µg/ ml)	OD	Mean	%Of Inhibition	%Of Viability	IC50 (µg/ml)
1	**Control**		**1.638**	**-**	**-**	**-**	**-**
2	**Standard (5, Flurouracil)**	25	1.123, 1.124, 1.123	1.123	31.44%	68.56%	58.62
		50	0.875, 0.876, 0.873	0.874	46.64%	53.36%	
		100	0.797, 0.795, 0.798	0.796	51.40%	48.6%	
		150	0.412, 0.411, 0.413	0.412	74.84%	25.16%	
		200	0.248, 0.248, 0.248	0.248	84.85%	15.15%	
3	***H. procumbens***	25	1.567, 1.568, 1.567	1.56	4.76%	95.24%	59.62
		50	1.110, 1.112, 1.113	1.111	32.17%	67.83%	
		100	0.797, 0.798, 0.796	0.797	51.34%	48.66%	
		150	0.699, 0.698, 0.699	0.698	57.38%	42.62%	
		200	0.589, 0.587, 0.588	0.588	64.10%	35.9%	

Figure 4: The microscopic pictogram shows effect of a.control, b.extract and c.standard on PC3 cell lines by MTT assay



a **b** **c**

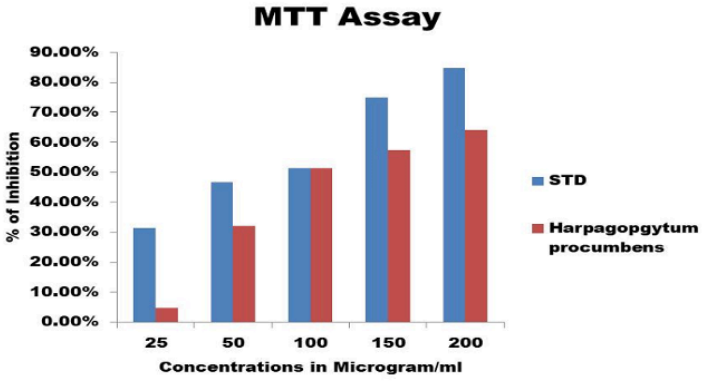
The untreated PC-3 cells used as the control exhibited typical epithelial-like morphology characterized by adherent growth, irregular polygonal shape, and distinct cell boundaries under microscopic observation (Figure 4a). The cells formed a monolayer with good confluency, confirming their healthy proliferative state. The identity and characteristics of the PC3 cell line were confirmed based on standard morphological features and adherence properties commonly reported for this prostate cancer cell line. These observations validate the suitability of the untreated cells as the control group for subsequent cytotoxicity evaluation.

Treatment with the standard drug 5-Fluorouracil induced significant morphological alterations in PC3, including cell shrinkage, loss of normal cell shape, and reduced cell density compared with the untreated control (Figure 4b). These structural changes indicate inhibition of cell proliferation and induction of cytotoxic effects in prostate cancer cells.

Treatment with the ethanolic root extract produced pronounced morphological changes in PC3, including cell shrinkage, membrane damage, and reduced cell density, indicating strong inhibition of cancer cell growth (Figure 4c). To exclude the possible effect of the solvent, a solvent control containing 0.2% DMSO/ethanol was included, which showed no significant morphological alterations compared to the untreated control. These observations confirm that the cytotoxic effect is attributed to the bioactive constituents present in the ethanolic extract rather than the solvent. The morphological analysis of PC3 prostate cancer cells revealed clear differences between treated and

untreated groups. Untreated control cells showed normal spindle-shaped morphology and dense growth. Cells treated with the standard extract displayed noticeable morphological changes such as cell shrinkage and reduced confluence, indicating inhibition of cancer cell proliferation. In contrast, cells treated with the ethanolic root extract of *Harpagophytum procumbens* exhibited more pronounced morphological alterations, including cell rounding and detachment, suggesting stronger cytotoxic and anticancer effects.

Figure 5: The graphical representation of *Harpagophytum procumbens* root extract on PC3 cell line by MTT Assay



The effect of *Harpagophytum procumbens* root extract ethanolic was performed by MTT assay on PC3 cell lines with concentration of 25,50,100,150,200µg ml and 5- flurouracil was used as a standard. the susceptibility of cell to the *Harpagophytum procumbens* root extract exposure was characterized by IC50values. The IC50 for the PC3 cell line was 59.62 µg/ml in ethanolic extract. Result indicates that the anticancer effect strengthens with increase in the concentration of drug. The MTT assay results indicate that both the ethanolic extract of *Harpagophytum procumbens* and the standard drug 5-Fluorouracil exhibited concentration-dependent cytotoxicity against PC3. However, 5-Fluorouracil produced higher percentage inhibition at lower concentrations, indicating a lower IC_{50} value and therefore greater potency compared to the plant extract. This stronger activity is expected because 5-Fluorouracil is a well-established chemotherapeutic agent that inhibits thymidylate synthase and disrupts DNA synthesis in rapidly proliferating cancer cells. Nevertheless, the extract of *Harpagophytum procumbens* also demonstrated notable cytotoxic activity, suggesting the presence of bioactive phytoconstituents with potential anticancer properties (Figure 5).

Conclusion

The present study demonstrated the anticancer potential of the ethanolic root extract of *Harpagophytum procumbens* through an integrated network pharmacology and in-vitro approach. Network pharmacology analysis identified key bioactive compounds such as rutin, harpagoside, β-sitosterol, and stigmasterol with favourable drug-likeness and ADMET properties, which were predicted to interact with important cancer-related targets including TP53, AKT1, VEGFA, EGFR, and MAPK1, suggesting modulation of multiple cancer-related signaling pathways. In-vitro evaluation using PC-3 confirmed that the extract exhibited concentration-dependent cytotoxic activity, although the standard chemotherapeutic drug 5-Fluorouracil showed comparatively higher potency.

Overall, the findings highlight the multi-target therapeutic potential of H. procumbens as a promising natural source for anticancer drug development. Future studies should focus on isolation of the active constituents, detailed molecular mechanism studies, and in-vivo validation to further confirm its efficacy and safety for prostate cancer therapy.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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