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Prediction of Rat Oral Toxicity, Genotoxicity and Toxicological Pathways of Selected Phytochemicals of *Cannabis sativa* Linn.: An *in silico* Approach

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Abstract: ***It was predicted acute toxicity (rat oral LD_{50}), genotoxicity, toxicological mechanisms of selected phytochemicals of Cannabis sativa. The 11 types of phytochemicals of leaf were selected as per literature.The ProTox-II webserver was used for predictive studies for toxicity and its mechanisms. The rat oral acute toxicity (LD_{50}) as mg/Kg, which predicted as class 4 and 6 toxicity class. Out of 11 compounds, six compounds viz. Delta-9-tetrahydrocannabinol, Cannabidiol, Cannabigerol, Cannabichromene, Oleic acid amide and Cannabidiphorol as Class 4 toxicity [prescribed harmful after swallowing (2000< LD_{50}≤5000)] while rest five compounds were predicted non-toxic as class 6. For hepatotoxicity, all the compounds were non-hepatotoxic as inactive. For immunotoxicity prediction, seven compounds viz. Delta-9-tetrahydrocannabinol, Cannabidiol, Cannabinol, Cannabichromene, Anandamide, N-Arachidonoyldopamine and Cannabidiphorol were immunotoxic. For genotoxicity end points, all the compounds were non-cytotoxic, non-carcinogenic and non-mutagenicas inactive. But Anandamide was predicted carcinogenic active. For nuclear receptor signalling pathway in which three compounds viz. Delta-9-tetrahydrocannabinol, Cannabinol and Cannabichromene were predicted AhR active. For AR and AR-LBD, all compounds were observed inactive. For Ar, two compounds viz. Delta-9-tetrahydrocannabinol, and Cannabichromene were found active. For ER, ER-LBD and PPAR-Y, all the studied compounds were inactive. For stress response pathway, one compound viz. 2-Arachidonoylglycerol was predicted ARE and HSE active. For McMP, eight compounds viz. Delta-9-tetrahydrocannabinol, Cannabidiol, Cannabinol, Cannabigerol, Cannabichromene, N-Arachidonoyldopamine, Oleic acid amide and Cannabidiphorol were observed active. For p53 and ATAD5, all compounds were predicted inactive. This predictive resultindicated overall toxicological mechanisms of phytocompounds of C. sativa, which helps in future experimental study.***

Keywords: *Cannabis sativa*, Predictive toxicology, Molecular mechanism of toxicity,Nuclear receptor signalling and stress response pathways

1. Introduction

Among several plant species, *Cannabis sativa* Linn. belonging to family Cannabaceae, also called as hemp ormarijuana, which has been used as medicinal herb earlier dates of 3000 BC (Turner et al., 1980). The seeds and leaves of the plant have therapeutic effects and its psychotropic resins have been used for medical, ritual, or spiritual purposes. [1]

On the other hand, marijuana or marihuana is referred to the leaves and flowering parts of cannabis as per usage of drug, intoxicant, or medicine. [2]Marijuana is primarily smoked or ingested orally when used for its psychoactive effects. [3]

The most important psychoactive constituent of marijuana is a cannabinoid, delta-9-tetrahydrocannabinol (THC), which produces relaxation, mild euphoria, sedation, and perceptual distortion. There are over 80 other cannabinoids including cannabidiol, cannabinol, and tetrahydrocannabivarin present in marijuana as well as THC.[3] It is necessary to evaluate the toxic phytochemicals, which can be detrimental impact after usage as narcotics or psychotropic agents.

Moreover, an *in silico* or virtual screening of these compounds are found inexpensive, less time consuming and no need to test with animal models. [4]In recent trend of *in silico* research, natural products or secondary metabolites from plants are showing main research interests for new inhibitory compounds of specific enzyme through traditional knowledge. [5-7]

The objective of the study was to predict acute toxicity (rat oral LD_{50}), genotoxicity, nuclear receptor signalling pathway and stress response pathway of selected phytochemicals of *Cannabis sativa*.

2. Materials and Methods

Selection of plant specimen

In the present predictive study, the plant specimen was selected as *Cannabis sativa* Linn. Under Cannabaceae family and this weed found in all parts of India. The phytochemicals of this tree have potential psychotropic or psychoactive agents

Selection of phytochemicals

The 11 types ofphytochemicals of leaf were selected as per earlier studies[6,8] and the two-dimensional (2D) structure of selected phytochemicals are exhibitedin Fig 1. These molecular structures were retrieved from ProTox-II web server.

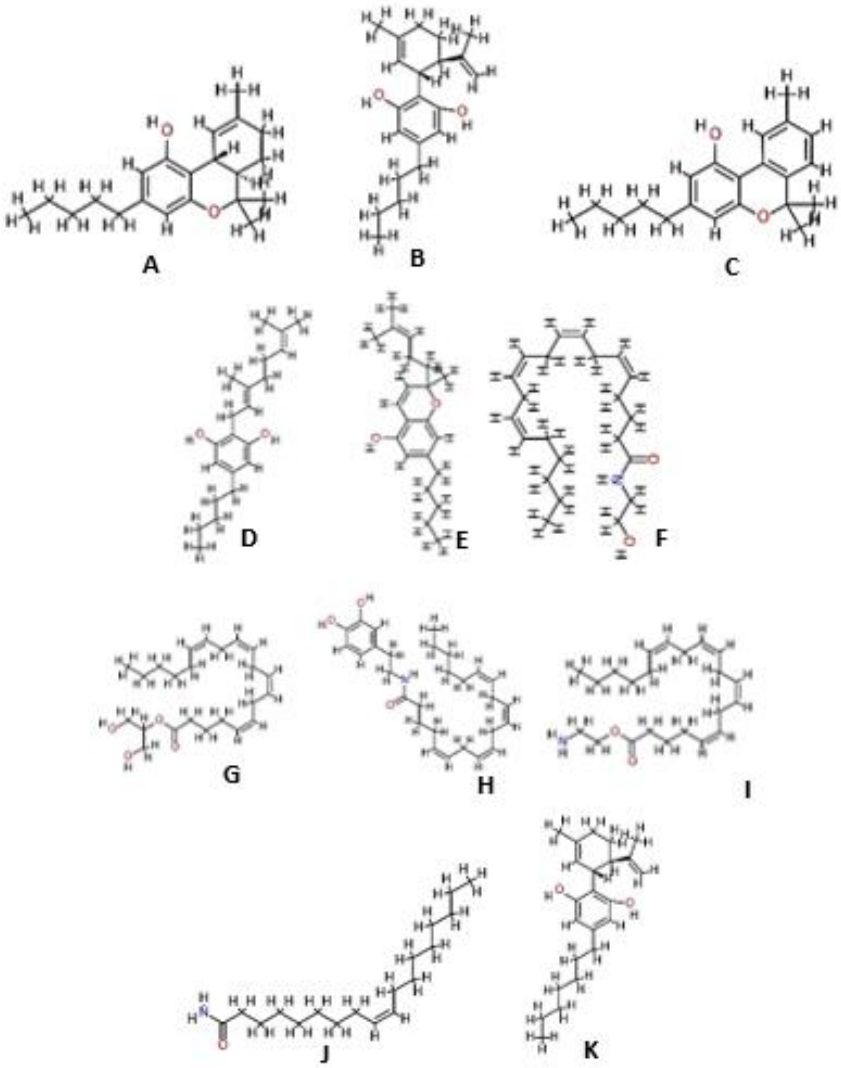

Figure 1: Two-dimensional structure of phytochemicals from *Cannabis sativa* [A = Delta-9-tetrahydrocannabinol; B = Cannabidiol; C = Cannabinol; D = Cannabigerol; E = Cannabichromene; F = Anandamide; G = 2-Arachidonoylglycerol; H = N-Arachidonoyldopamine; I = O-Arachidonoylethanolamine; J = Oleic acid amide and K = Cannabidiphorol]

Prediction of toxicity

The toxicity screening especially rat's oral acute toxicity to know median lethal dose (LD_{50}) as mg/Kg (the lethal dose at which 50% of the test model dies) and hepatotoxicity, immunotoxicity, cytotoxicity, mutagenicity, and carcinogenicity as well as toxicological mechanisms under nuclear receptor signalling pathway and stress response pathway werepredicted by using ProTox-II web server developed by Drwal et al.[9]and protocol established by Banerjee et al.[10]

3. Results

Table 1tabulates the rat oral acute toxicity (LD_{50}) as mg/Kg, which predicted as class 4 and 6 toxicity class and prediction accuracy (%) for different selected compounds of *C. sativa*. Out of 11 compounds, six compounds viz. Delta-9-tetrahydrocannabinol, Cannabidiol, Cannabigerol, Cannabichromene, Oleic acid amide and Cannabidiphorol as Class 4 toxicity [prescribed harmful after swallowing ($2000<LD_{50}\leq5000$)] while rest five compounds were predicted non-toxic as class 6. Dose-response curve of each phytocompound is exhibited in Fig 2.

Table 1: Prediction of rat oral toxicity for phytochemicals of *C. sativa*

Sl. No.	Phytochemicals	Rat oral LD_{50} (mg/Kg)	Toxicity class	Predictive accuracy (%)
1.	Delta-9-tetrahydrocannabinol	482	4	100.00
2.	Cannabidiol	500	4	68.07
3.	Cannabinol	13500	6	100.0
4.	Cannabigerol	500	4	67.38
5.	Cannabichromene	750	4	70.97
6.	Anandamide	50000	6	69.26

7.	2-Arachidonoylglycerol	20000	6	70.97
8.	N-Arachidonoyldopamine	1000	4	60.07
9.	O-Arachidonoylethanolamine	20000	6	70.97
10.	Oleic acid amide	750	4	69.26
11.	Cannabidiphorol	500	4	68.07

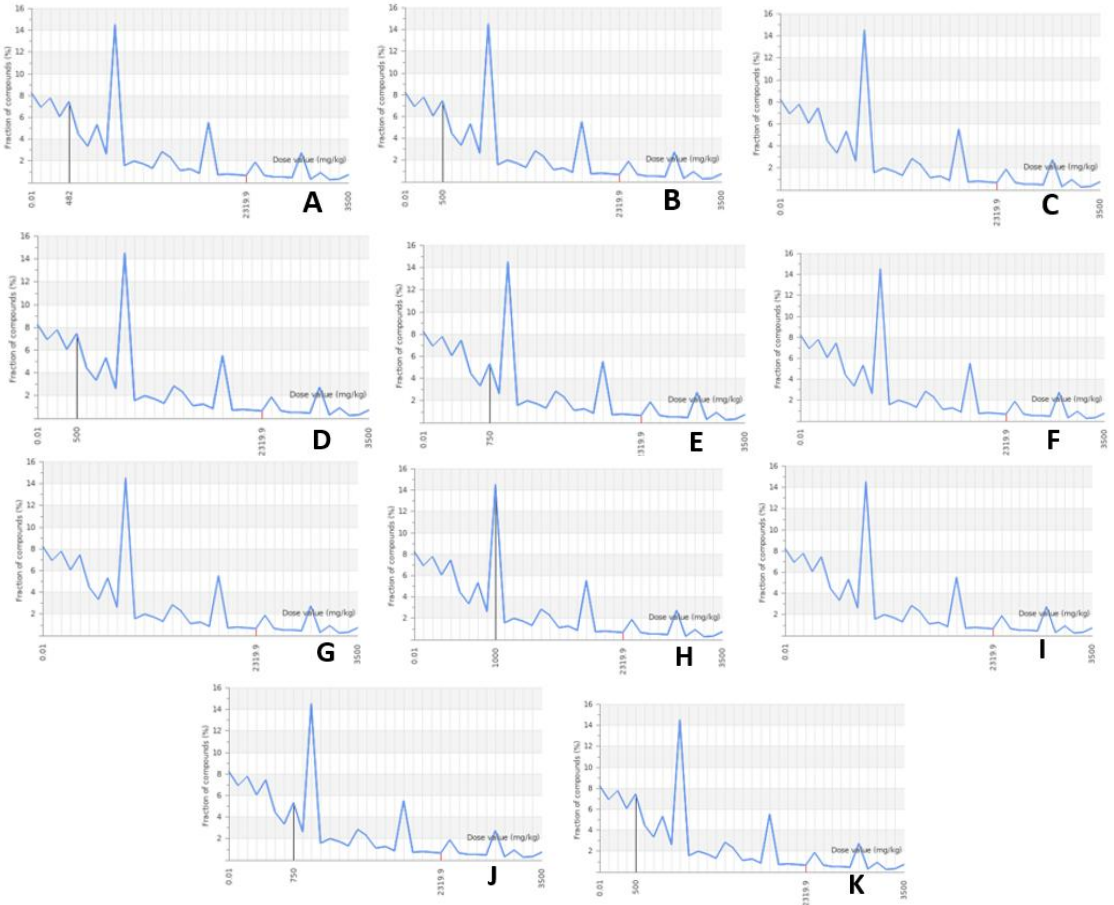

Figure 2: Dose-response curve of phytochemicals from *C. sativa*[A = Delta-9-tetrahydrocannabinol; B = Cannabidiol; C = Cannabinol; D = Cannabigerol; E = Cannabichromene; F = Anandamide; G = 2-Arachidonoylglycerol; H = N-Arachidonoyldopamine; I = O-Arachidonoylethanolamine; J = Oleic acid amide and K = Cannabidiphorol]

Table 2 tabulates the prediction of organ toxicity especially liver toxicity or hepatotoxicity as well as immunotoxicity. In the case of hepatotoxicity, all the compounds were non-hepatotoxic as inactive. For immunotoxicity prediction, seven compounds viz. Delta-9-tetrahydrocannabinol, Cannabidiol,Cannabinol,Cannabichromene,Anandamide, N-Arachidonoyldopamineand Cannabidiphorol were immunotoxic and remaining 4 compounds were immunotoxic inactive.

Table 2: Prediction of liver toxicity and immunotoxicity for phytochemicals of *C. sativa*

Sl. No.	Phytochemicals	Hp	P (%)	Im	P (%)
1.	Delta-9-tetrahydrocannabinol	I	93.0	A	99.0
2.	Cannabidiol	I	79.0	A	93.0
3.	Cannabinol	I	87.0	A	73.0
4.	Cannabigerol	I	83.0	I	67.0
5.	Cannabichromene	I	91.0	A	95.0
6.	Anandamide	I	76.0	A	0.50
7.	2-Arachidonoylglycerol	I	91.0	I	95.0
8.	N-Arachidonoyldopamine	I	83.0	A	71.0
9.	O-Arachidonoylethanolamine	I	85.0	I	92.0
10.	Oleic acid amide	I	82.0	I	98.0
11.	Cannabidiphorol	I	82.0	A	96.0

Hp = Hepatotoxicity; Im = immunotoxicity; I = Inactive; A = Active; P = Probability

Table 3 tabulates the prediction of genotoxicity end points such as cytotoxicity, carcinogenicity, and mutagenicity. All the compounds were non-cytotoxic, non-carcinogenic and non-mutagenic as inactive. But Anandamide was predicted carcinogenic active.

Table 3: Prediction of cytotoxicity, carcinogenicity and mutagenicity for phytochemicals of *C. sativa*

S. No.	Phytochemicals	Ct	P (%)	Cr	P (%)	Mt	P (%)
1.	Delta-9-tetrahydrocannabinol	I	84.0	I	86.0	I	77.0
2.	Cannabidiol	I	87.0	I	66.0	I	85.0
3.	Cannabinol	I	86.0	I	81.0	I	69.0
4.	Cannabigerol	I	87.0	I	77.0	I	78.0
5.	Cannabichromene	I	85.0	I	82.0	I	76.0
6.	Anandamide	I	76.0	A	51.0	I	93.0
7.	2-Arachidonoylglycerol	I	84.0	I	59.0	I	80.0
8.	N-Arachidonoyldopamine	I	77.0	I	59.0	I	65.0
9.	O-Arachidonoylethanolamine	I	71.0	I	63.0	I	87.0
10.	Oleic acid amide	I	72.0	I	60.0	I	93.0
11.	Cannabidiphorol	I	88.0	I	66.0	I	84.0

Ct = Cytotoxicity; Cr = Carcinogenicity; Mt = Mutagenicity; I = Inactive; A = Active; P = Probability

Table 4 tabulates the prediction of nuclear receptor signalling pathway in which three compoundsviz. Delta-9-tetrahydrocannabinol, Cannabinol and Cannabichromene were predicted AhR active while rest compounds were found inactive. For AR and AR-LBD, all compounds were observed inactive. For Ar, two compoundsviz. Delta-9-tetrahydrocannabinol, and Cannabichromene were found active while rest compounds were observed inactive. For ER, ER-LBD and PPAR-ϒ, all the studied compounds were inactive.

Table 5 tabulates the prediction of stress response pathway in which one compoundviz. 2-Arachidonoylglycerol was predicted ARE and HSE active while rest compounds were found inactive. For McMP, eight compounds such as Delta-9-tetrahydrocannabinol, Cannabidiol, Cannabinol, Cannabigerol, Cannabichromene, N-Arachidonoyldopamine, Oleic acid amide and Cannabidiphorol were observed active while remaining three compounds were found inactive. For p53 and ATAD5, all compounds were predicted inactive.

Table 4: Prediction of nuclear receptor signalling pathways for phytochemicals of *C. sativa*

Sl. No.	Phytochemicals	AhR	P (%)	AR	P (%)	AR-LBD	P (%)	Ar	P (%)
1.	Delta-9-tetrahydrocannabinol	A	100.0	I	98.0	I	99.0	A	100.0
2.	Cannabidiol	I	57.0	I	96.0	I	98.0	I	79.0
3.	Cannabinol	A	99.0	I	99.0	I	97.0	I	66.0
4.	Cannabigerol	I	73.0	I	98.0	I	99.0	I	91.0
5.	Cannabichromene	A	72.0	I	95.0	I	97.0	A	93.0
6.	Anandamide	I	99.0	I	99.0	I	99.0	I	100.0
7.	2-Arachidonoylglycerol	I	1.0	I	99.0	I	99.0	I	99.0
8.	N-Arachidonoyldopamine	I	98.0	I	99.0	I	1.0	I	91.0
9.	O-Arachidonoylethanolamine	I	96.0	I	99.0	I	99.0	I	1.0
10.	Oleic acid amide	I	99.0	I	1.0	I	1.0	I	1.0
11.	Cannabidiphorol	I	65.0	I	96.0	I	98.0	I	81.0
Sl. No.	Phytochemicals	ER	P (%)	ER-LBD	P (%)	PPAR-ϒ	P (%)		
1.	Delta-9-tetrahydrocannabinol	I	85.0	I	96.0	I	97.0		
2.	Cannabidiol	I	62.0	I	93.0	I	93.0		
3.	Cannabinol	I	78.0	I	93.0	I	91.0		
4.	Cannabigerol	I	50.0	I	83.0	I	91.0		
5.	Cannabichromene	I	84.0	I	96.0	I	97.0		
6.	Anandamide	I	86.0	I	99.0	I	98.0		
7.	2-Arachidonoylglycerol	I	98.0	I	99.0	I	99.0		
8.	N-Arachidonoyldopamine	I	94.0	I	98.0	I	99.0		
9.	O-Arachidonoylethanolamine	I	87.0	I	98.0	I	95.0		
10.	Oleic acid amide	I	98.0	I	99.0	I	99.0		
11.	Cannabidiphorol	I	58.0	I	75.0	I	92.0		

AhR = Aryl hydrogen receptor; AR = Androgen receptor (AR); AR-LBD = Androgen receptor ligand binding domain; Ar = aromatase; ER Estrogen receptor alpha; ER-LBD = Estrogen receptor ligand binding domain and PPAR-ϒ = peroxisome proliferator activated receptor gamma; I = Inactive; A = Active; P = Probability

Table 5: Prediction of stress response pathways for phytochemicals of *C. sativa*

S. No.	Phytochemicals	ARE	P (%)	HSE	P (%)	McMP	P (%)	p53	P (%)	ATAD5	P (%)
1.	Delta-9-tetrahydrocannabinol	I	83.0	I	83.0	A	0.99	I	91.0	I	98.0
2.	Cannabidiol	I	65.0	I	65.0	A	0.83	I	75.0	I	97.0
3.	Cannabinol	I	71.0	I	71.0	A	96.0	I	87.0	I	96.0
4.	Cannabigerol	I	79.0	I	79.0	A	89.0	I	83.0	I	98.0
5.	Cannabichromene	I	84.0	I	84.0	A	75.0	I	90.0	I	98.0
6.	Anandamide	I	94.0	I	94.0	I	91.0	I	99.0	I	100.0
7.	2-Arachidonoylglycerol	A	100.0	A	100.0	I	99.0	I	99.0	I	99.0
8.	N-Arachidonoyldopamine	I	97.0	I	97.0	A	1.0	I	98.0	I	99.0
9.	O-Arachidonoylethanolamine	I	79.0	I	79.0	I	91.0	I	97.0	I	99.0

10.	Oleic acid amide	I	98.0	I	98.0	A	96.0	I	98.0	I	1.0
11.	Cannabidiphorol	I	66.0	I	66.0	A	81.0	I	74.0	I	97.0

ARE = Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element; HSE = Heat shock factor response element; McMP = Mitochondrial membrane potential; p = 53 = Phosphoprotein tumor suppressor; ATAD5 = ATPase family AAA domain-containing protein 5; I = Inactive; A = Active; P = Probability

4. Discussion

In this *in silico* study, the prediction was performed on acute toxicity (rat oral LD_{50}), genotoxicity, nuclear receptor signalling pathway and stress response pathway of selected phytochemicals of *C. sativa*. Many investigators reported the cannabis-induced adverse effects that may be influenced by several factors such as genetic variation, age, sex, ethnicity, and duration and frequency of cannabis use. [8,11-13]

Moreover, in children, cannabis-induced symptoms were also observed.[14]Fortunately, these toxicity indications are usually occurred rapidly and last for long-term effect. [14,15]On the other hand, THC was detected in the product ingested by the child, and the acid metabolite of THC was detected in the child's urine. [14,16]Carstairs et al. [17]reported a case of a 14-month-old child who ingested hashish and was in a prolonged coma for more than 48 hours. The THC metabolite, 11-nor-carboxy-Δ9-THC, was detected in high levels in the child's urine, and the clinical cureapprovedafter declining the level of THC metabolite in urine. [17]

Cannabis intoxication is dose-related, and its absorbance is dependingupon the route of administration and concentration based. Inhaled doses of 2-3 mg and ingested doses of 5-20 mg of THC can affect memory and cause short-term memory impairment and loss of attention, while inhaled doses more than 7.5 mg/m^2 in adults and oral doses of 5-300 mg in children can cause more serious effects, such as respiratory depression, panic, anxiety, hypotension, myoclonic jerking, and other symptoms. [8,18]The LD_{50} of THC is not determined in humans due to ethical causes, but in animals it ranges from 40-130 mg/kg intravenously. The LD_{50} of THC inhalation from smoked cannabis in Fisher rats is 42 mg/Kg, a value that is similar to the intravenous vascular access port value, which indicates that THC is the active intoxicant of smoked cannabis. [8,18,19]But in present *in silico*study, Delta-9-tetrahydrocannabinol predicted 484 mg/Kg oral LD_{50} of rat.

Regarding the toxicological mechanisms especially nuclear signalling pathway and stress response pathway few phytocompounds were predicted active and some were inactive. Till date, experimental studies are to be needed to know toxicological mechanisms. Morales et al. [20]reported that the same phytocannabinoid working at multiple targets in which phenomena are much more complex. It is well-known that mitochondria comprise double membrane, which help to create the energy to the cell through oxidative phosphorylation and prevent apoptosis. [21]While mitochondrial stress by toxins may lead to several diseases. [22] Interestingly, Richter et al. [23]reported that toxins have capability to inhibit the mitochondrial protein synthesis and block with the stress response. In the present predictive study, eight compounds such as Delta-9-tetrahydrocannabinol, Cannabidiol, Cannabinol, Cannabigerol, Cannabichromene, N-Arachidonoyldopamine, Oleic acid amide and Cannabidiphorolwere observed active for McMP, which indicated these phytocompounds may have deleterious impact on mitochondria.

5. Conclusion

It is concluded from the above predictive results that few phytocompounds of *C. sativa* observed toxic, immunotoxic and one of these carcinogenic, and disruptor of McMP. The present *in silico* findings is suitable for further experimental research in which toxic phytocompound were obtained in a narrow range. This *in silico* study also helps in faster screening of phytocompounds. This study is suggested future experimental assay viz. *in vivo* and *in vitro* to validate the present prediction of studied phytocompounds.

Acknowledgment
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Conflict of interest
As per authors, it is declared no conflict of interest.

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