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ARTICLE

CHEMOSENSITIZING EFFECTS OF FIVE PHYTOCHEMICAL COMPOUNDS ON CANCER CELLS

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ABSTRACT

Background: *Increasing number of natural products have been used for cancer treatment. More and more pure components from natural products have identified with beneficial effect including both direct cytotoxic effect and chemosensitizing effect (CE). The cytotoxicity indicates a potential use for inhibiting tumor growth, while CE can be applied to overcome the chemoresistance.* ***Methods:*** *In this study, five active components including gallic acid, tannic acid, quercetin, myrecitin and serotonin widely studied for their activity in improving human health, were tested for the cytotoxicity as well as CE against prostate, leukemic and breast cancer cells.* ***Results:*** *These compounds were cytotoxic effect if administered alone, while they showed chemosensitizing effect (CE) on current chemotherapeutic drugs.* ***Conclusions:*** *This may represent a new pharmacological strategy to treat several types of cancer cells by providing mono- or multi-therapies that are significantly reduces the risk of anticancer side effect.*

KEY WORDS
Cytotoxicity, Tannic acid, Quarecitine, Myrecitine, Qallic acid, Serotonine and Chemosenstizing Effect.

INTRODUCTION

Cancer is a leading cause of death worldwide and can be induced by many factors [1], such as exposure to exogenous sources including the reactive oxygen species, nitrogen oxide pollutants, smoking, certain drugs (e.g, acetaminophen, bleomycin), and radiation. Other components affecting signal transduction pathways leading to uncontrolled cell proliferation may also increase the risk of cancer [2, 3]. Herbal medicines have been frequently used for cancer treatment as well as prevention [4,5,6].

Chemotherapy is one of the most frequently used approaches for cancer treatment. However 90% of patients would experience chemoresistance leading to therapeutically failure. The drug combination of a chemotherapeutic agent with or a few natural products has been widely studied to achieve synergistic effect which may enhance the drug efficacy but reduce side effect [7,8].

In this study, we focused on tannic acid, quercetin, myrecitin, gallic acid and serotonin which are phytochemical compounds. Their toxicity as well as combination effect with marketed therapeutic agents (docetaxel and daunorubicin) in various cancer cells were determined.

MATERIALS AND METHODS

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Reagents and Cell Lines

The human prostate cancer cell line, PC3 and the corresponding docetaxel resistant cell line PC3-TxR were kindly provided by Department of Medicine, University of Pittsburgh and Partners Healthcare (Pittsburgh, PA, USA). The human leukemic cancer cell line K562 and its daunorubincin resistant cell line (K562/Dox) were obtained from Western University of Health Science, College of Pharmacy (Pomona, CA USA). The breast cancer cell line (MCF7) was purchased from ATCC (ATCC, Manassas, VA, USA).
RPMI 1640 medium, glutamine, trypsin-EDTA, and fetal bovine serum were obtained from Cellgro (Manassas, VA, US) and Invitrogen (Grand Island, NY, US). Sulforhodamine B, trichloroacetate acid, and Tris base were bought from Sigma-Aldrich (St. Louis, MA, US). Quercetin, myrecitin, tannic acid, gallic acid and serotonin obtained from Sigma-Aldrich (St. Louis, MA, US).

Cell lines and Cell Culture

The human prostate cancer cell lines PC3 and PC3-TxR, human Leukemic cancer cell lines K562 and K562-Dox and breast cancer cell line (MCF7), were cultured in a humidified atmosphere 5 % CO_2 at 37˚C in RPMI-1640, supplemented with 10% heat inactivated fetal bovine serum, and 100 IU/ml of penicillin and 100 µg/ml of streptomycin. Cells were kept in the logarithmic phase by routine passage every 2-3 days using 0.05% trypsin-EDTA treatment.

Cells were then seeded into 96-well plate at densities of $3x10^3$ cell/well for PC3, PC3-TxR and MCF7, while $10x10^3$ cell/well for K562 and K562-Dox The cells were incubated at 37˚C (5% CO_2) overnight to allow

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attachment onto the wells [9], after 24 hr added 100 µL of different phytochemical compounds concentrations in range ($1x10^{-3}$-$2x10^{-3}$) mg/ml for quercetin, tannic acid, gallic acid and serotonin, while ($0.5x10^{-3}$-$1x10^{-3}$) mg/ml for myrecitin, following incubation at 37˚C in an atmosphere of 5% CO_2 for 72hr, then SRB assay was performed. Briefly, the cells were fixed with 10% trichloroacetic acid solution for prostate and breast cancer cell lines while leukemic cancer cell lines were fixed with 80% trichloroacetic acid. All cell lines incubated for one hour at 4˚C, washed 3-4 times with tap water, and dried in the air. Cells were stained with 0.4% SRB, and then washed with 1% acetic acid solution after dry; dissolve the cell stain with 10mM Tris (PH 10.0) and absorbance was measured at 565nM and 515nM by UV-plate reader [10][25].
IC50 was calculated using Emax sigmoid method with aid of computer software, Graphpad prism (San Diego, CA, USA).

Chemosensitizing study

Prostate cancer cells which sensitive and resistant to docetaxel (PC3 and PC3-TxR), and leukemic cancer cells sensitive and resistant to daunorubicin (K562 and K562-Dox) cell lineswere cultured in volume 100µL culture medium and incubated for 24 hr of incubated at 37˚C in 5% CO_2 (In replicate). Afterwards, 50 µL of phytochemical compounds at concentration range ($o.35x10^{-3}$-$5.7x10^{-3}$) mg/ml was added to top half of 96-well plate, after one hour incubated, docetaxel or daunorubicin in different concentrations were added to final concentration ranged from 0 to 100 nM and 0-100 µM for docetaxel and daunorubicine respectively. All plates incubated at 37˚C in (5% CO_2), after incubation for another 72 hours, the cell viability was determined using an SRB assay and Inhibition Concentration (IC_{50}) calculated using a sigmoid Emax model [11][26]. The CE was calculated using the following equation [12].

$$\text{Chemosenstizing Effect (CE)} = IC_{50}\ (\text{Drug})\ /IC_{50}\ (\text{drug combination}).$$

Where IC50(Drug) is the IC50 of drug (docetaxel or daunorubicin) alone; IC50 (drug combination) is the corresponding IC50 of drug in combination with herbal substance).

RESULTS

The IC50 of phytochemicals compounds in human cancer cells lines are shown in [Table 1]

The cytotoxicity of quercetin, myrecitin, tannic acid, gallic acid and serotonin is varied in these five cell lines. Among these compounds, tannic acid, myrecitin, serotonin, and gallic acid are relative toxic to prostate cancer and leukemia cell lines. All of these compounds are not effective in inhibition of breast cancer cells with IC50>50 µg/ml.

Table 1: Inhibitory Concentration (IC_{50}) of different phytochemicals compounds on cancer cell lines

	IC_{50} (µg/ml)				
compounds	**MCF7**	**PC3**	**PC3-TxR**	**K562**	**K562-Dox**
Quercetin	677.0±32.52	19.7±1.31	95.5±9.19	65.05±7.14	57±12.72
Myrecitin	475.5±36.06	21.0±1.41	19.65±2.05	19.8±3.11	10.2±1.55
Tannic acid	262.5±55.86	2.15±0.21	9.85±2.05	1.00±0.56	0.75±0.21
Gallic acid	142.77±279.52	2.15±0.07	20.05±4.31	18.90±1.55	2.75±0.49
Serotonin	86.33±164.96	1.00±0.28	10.15±1.48	20±4.38	1.51±0.69

Chemosensitizing effect of phytochemical compounds on cancer cells line

Activity of gallic acid, serotonin, quercetin, myrecitin and tannic acid showed significant effect on prostate cancer cell which are resistant to docetaxel (PC3-TxR) with CE values (1.19±0.0262, 1.397±0.0211, 1.679±0.0242, 1.125±0.058 and 1.091±0.0262) nM respectively, as [Fig. 1].

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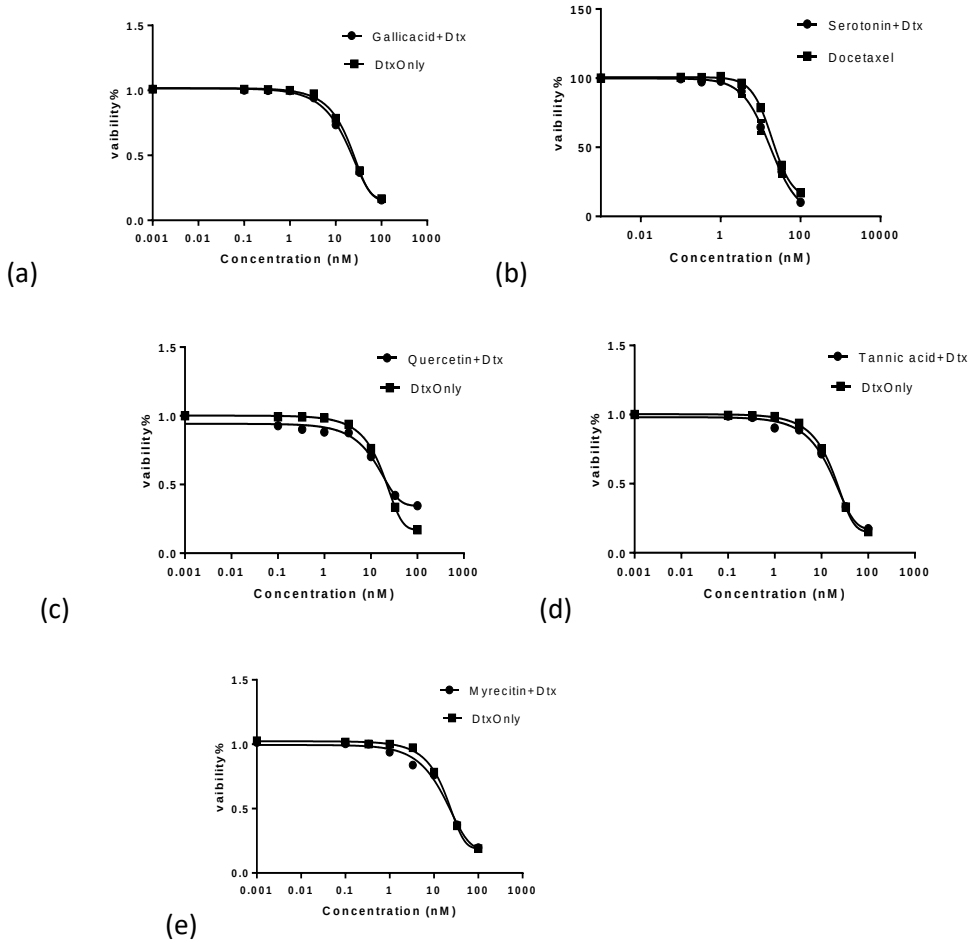
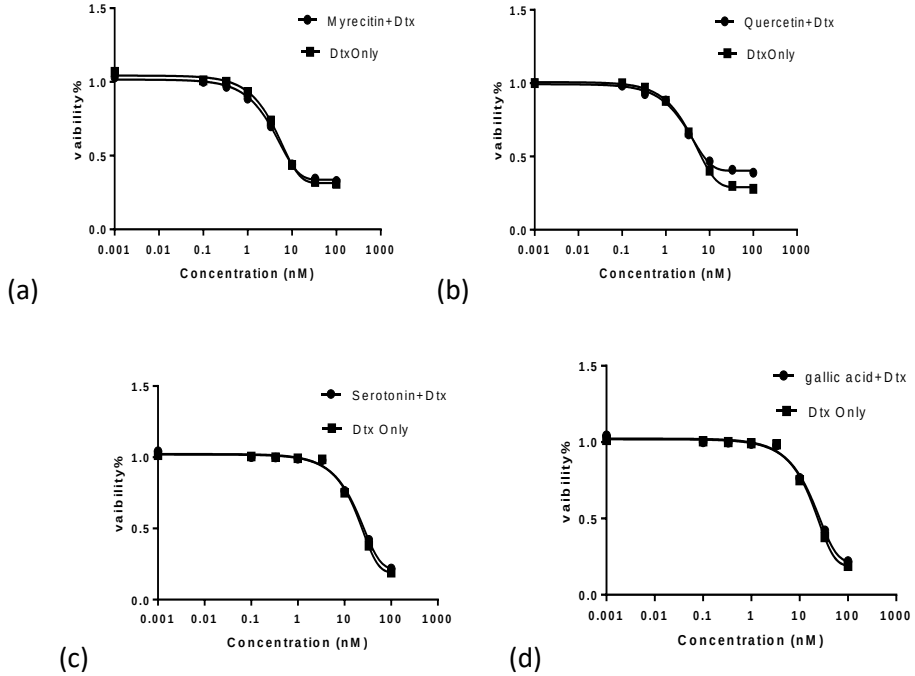


Fig. 1: Chemosensetizing effect of phytochemical compounds (a) Gallic acid, (b) Serotonin, (c) quercetin, (d) Tannic acid and (e) Myrecitin, on prostate cancer cells resistance to docetaxel (PC3-TxR).

While chemosensitizing effect on sensitive prostate cancer cell lines have shown no significant effects, as shown in [Fig. 2].



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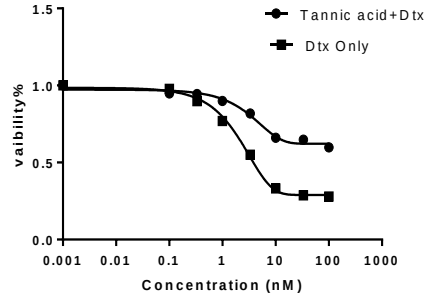
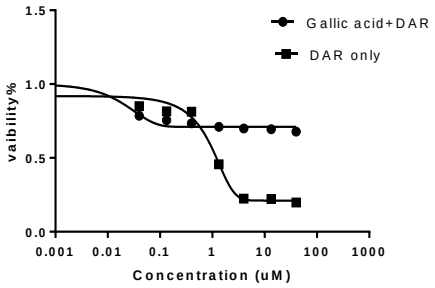


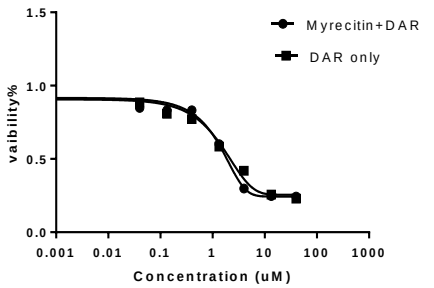
(e)

Fig. 2: Chemosensitizing effect of phytochemical compounds (a) Myricitin, (b) Quercetin, (c) Serotonin, (d) Gallic acid and (e) Tannic acid, on sensitive prostate cancer cell lines (PC3).

The chemosensitzing effect of serotonin, myricitin, gallic acid, qurecetin and tannic acid are shown in [Fig. 3]. No significant effect on sensitive leukemic cancer cells (K562) was observed. However for the resistant cell lines, K562/Dox resistant to danuorubicin, serotonine (CE= 4.006±0.119)uM displayed a substantial chemosenstizing effect in camparison to tannic acid (CE= 0.926±0.081)uM, gallic acid (CE= 0.895±0.111)uM, myrecitin (CE= 1.035±0.07)uM and guercetin (CE= 1.076±0.045)uM, [Fig. 4].



(a)



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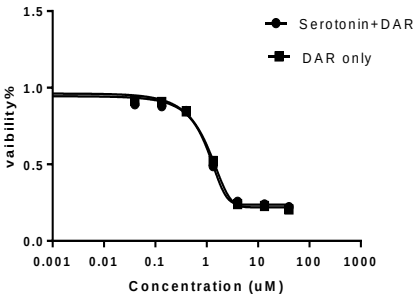

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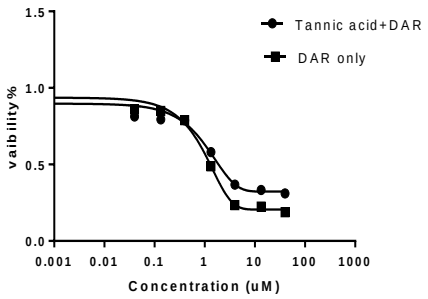

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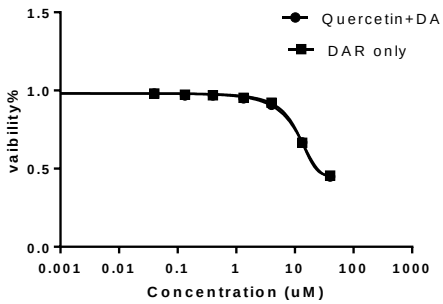

(e)

Fig. 3: Chemosensitizing effect of phytochemical compounds (a) gallic acid (b) myrecitin, (c) serotonin, (d) tannic acid, and (e) quercetin, on leukemic cancer cell lines which are sensitive to daunorubicin (K562).

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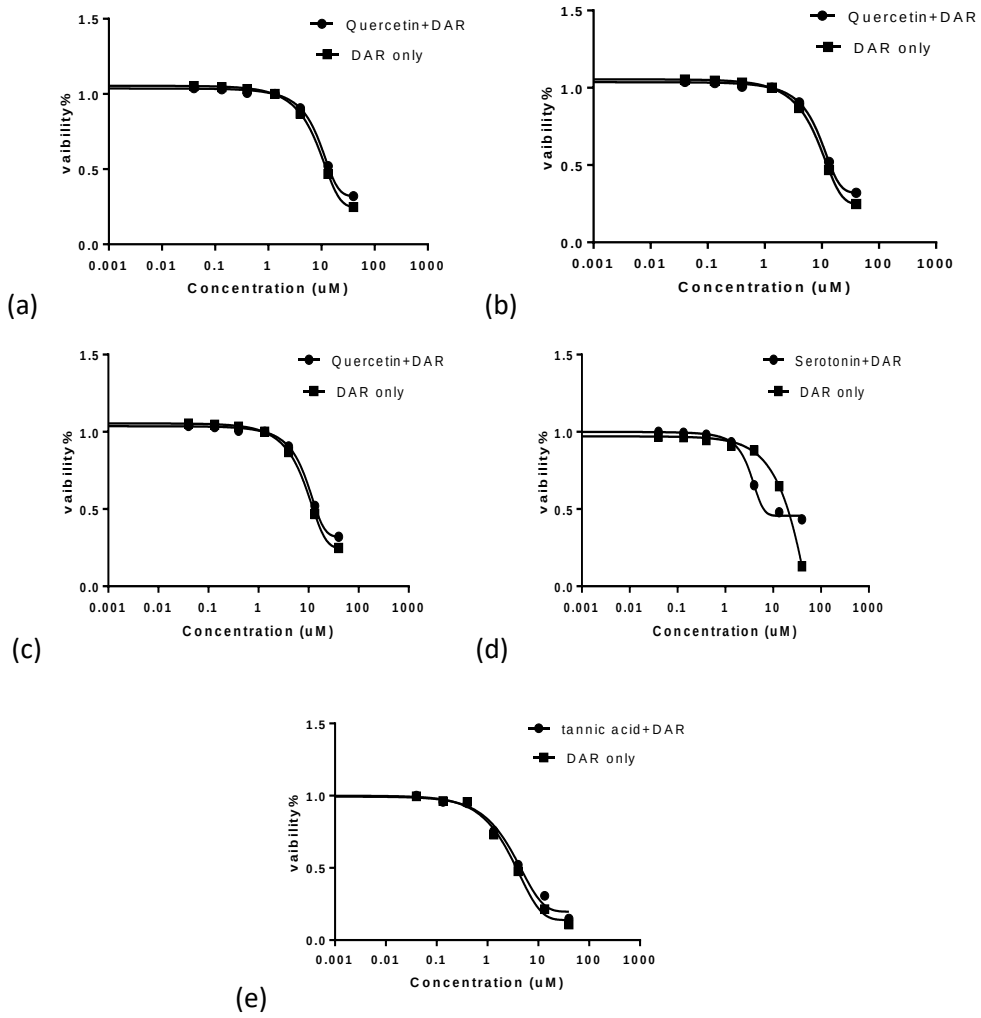

Fig. 4: Chemosensitizing effect of phytochemical compounds (a) myrecitin, (b) gallic acid, (c) quercetin, (d) serotonin and (e) tannic acid, on leukemic cancer cell lines which are resistant to daunorubicin (K562/Dox).

When combining phytochemical compounds (gallic acid, tannic acid, myrecitin, quercetin and serotonin) together, the combination did not show any chemosensitizing effect on leukemic cancer cell that are resistant to daunurubicin (K562/Dox) (CE= 0.885±0.032)uM and prostate cancer cell that are resistant to docetaxel (PC3-TxR) (CE= 0.901±0.011)µM, as [Fig. 5].

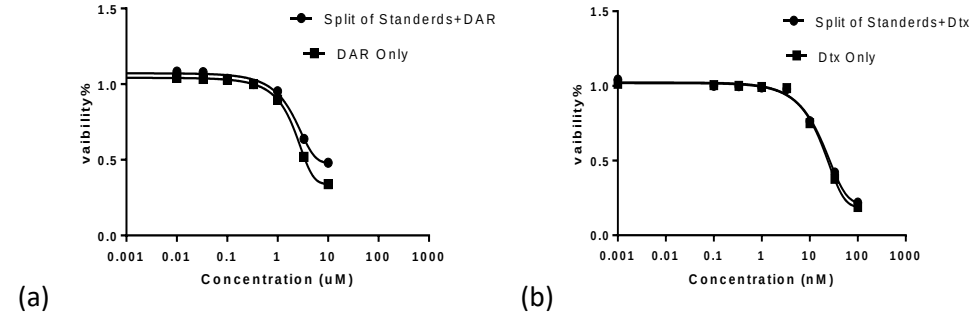

Fig. 5: Chemosensitizing of Split standards pure compounds on (a) K562dox and (b) PC3TxR cancer cells.

DISCUSSION

In this study, we had demonstrated the cytotoxic and chemosensetizing effects of five phytochemical compounds (myrecitin, quercetin, gallic acid, tannic acid and serotonin) on prostate cancer cells (PC3 and

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PC3TxR), leukemic cancer cells (K562 and K562Dox) and breast cancer cells, which showed significant inhibition of growth cancer cells if they were administrated alone or when combined with chemotherapeutic drug, specially on resistance cancer cells.

These phytochemical compounds have been reported a broad range of pharmacological effects, including anti-oxidant and anti-inflammatory activities [13], as well as, have been associated with anti-proliferative effects [14] and anti-cancer agent for current cancer therapies [15].

The antioxidant mechanisms of these phytochemical compounds are the induction of apoptosis in cancer cells and prevention of ongiogenesis and metastatic spread. These effects are suggesting a potential role for antioxidants as adjuvant in cancer therapy and have pharmacological actions like prooxidant toxicity and apoptosis, with reducing painful side effect associated with treatment [16,17], as well as, possessing the potential role to scavenge and quench various radicals (oxygen-centered, carbon-centered, alkoxyl, peroxyl, or phenoxyl redicals) and ROS [18,19,20].

Sara *et al*., 2012, suggested the natural products which derived from plants may provide solve for many problems like; lack of success with targeted mono- therapy and drug resistance which result from continuing use of chemotherapeutic agents.

The drug resistant mechanisms of cancer cells are the existence of subpopulations of cancer cell through the cellular interactions that impaired drug delivery to the cancerous cells. Chemosensitizing effects of phytochemical compounds to regimens chemotherapeutic drugs would be the way to go in order to increase the cytotoxic effect at a given dosage concentration while minimizing side effect [22,23]. However, most of the cells do not showed resistance to natural plant products, therefore, they may provid alternative modality of treatment for multidrug resistant tumors [24].

CONCLUSION

In summary, the safety and independent anticancer effect of these compounds support the use of them as an adjunct to chemotherapy which could be used as mono- or multi-therapies in the treatment of cancer cells.

CONFLICT OF INTEREST

There is no conflict of interest.

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FINANCIAL DISCLOSURE

None

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