Chemoprotective effect of *Melastoma malabathricum* Linn Against Diethylnitrosamine (DENA) Induced Cancer

Vipin Kesharwani¹*, Manoj Kumar Mishra¹, Kuldeep singh¹, Nikhil Kushwaha¹, Sudha Kumari¹

¹Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj, Uttar-Pradesh, India

**ARTICLE INFO**

**ABSTRACT**

**Objective:** The present study was aimed to Chemoprotective Effect of *Melastoma Malabathricum* Linn against Diethylnitrosamine (DENA) Induced Cancer.

**Method:** The leaves were chopped to smaller pieces and were thoroughly washed, dried under room temperature. By using Wiley mill, dried leaves were powdered. Accurately 100 gm of powdered leaves was weighed and transferred in a Soxhlet apparatus and extraction was performed using ethanol. Further, the extracts were concentrated using rotary evaporator, which was used for preliminary phytochemical screening as well as for anticancer activity.

**Result:** The result of Hepatic cancer of extract leaves of *Melastoma Malabathricum* on DENA Induced Hepatic toxicity in rat. The liver marker enzyme, Hb, TLC, ESR, SGOT, SGPT blood serum and EDTA significantly rise level in DENA toxic group treated animals while comparing with normal/control group.

**Conclusion:** From the detailed investigation on the present study inferred so far pertaining to MM effect on hepatoprotective activity from the ethanolic extracts of *Melastoma Malabathricum Linn*. Thus from the inferred study presented, it represented hepatoprotective activities showcased by extract of ethanolic *Melastoma Malabathricum* Linn. Leaves of DENA-induced hepatotoxicity showed significant improvement in liver enzymes level.
INTRODUCTION:
Cancer is one such disease which causes abnormal proliferation of cell and also has the potential towards spreading/invading to other body parts. Such abnormalities generally result in the eventual death of the individuals. Cancer has become one among the life threatening medical conditions which requires a standard and efficient therapeutic intervention for treating it completely. Many researchers are in the verge of discovering a suitable therapeutically active compound that is naturally derived from the marine/terrestrial floral and faunal species (Schumacher M et al., 2001). Cancer is regarded as a major class of disease which is primarily characterized by uncontrollable cell growth. It was identified that nearly 100 different forms of cancer types are presently classified. These cancer types were basically differentiated based totally at the beginning and type of cell (Hiatt H et al., 1997). Cancer affects the bodily system by division of body cells in uncontrollable manner resulting in the formation of lumps and tissue masses which is otherwise called tumors. Cancer causes over 2-3 % deaths globally from the annual reports. Furthermore, the reports from American Cancer Society (ACS) stated that among the 14.1 million people were newly diagnosed with cancer cases, of which 8.2 million died in the year 2012 and it is expected that around 21.7 million will eventually be diagnosed with cancer by 2030, and 13 million are expected to be in their terminal stage of cancer (Edge S.B 2010). There are numerous factors pertaining with lifestyle issues that are primarily responsible for cancer risks viz smoking, physical inactivity, poor diet and minimal pregnancies especially in developing countries American cancer society. When the cancer reaches the metastasis stage, the individuals are highly susceptible for treatment as it is quite difficult for treating. Metastasis has been accounting to be the most prominent reason for most of the cancer deaths and is a unique for the researchers in controlling and treating the metastases. This could be mainly due to their
- Smaller size
- Higher multiplicity potential
- Overall dispersion to the vital organs and body parts

Hepatic Cancer
Liver cancer or otherwise referred Hepatocellular Carcinoma (HCC) which in general, is regarded as upmost dangerous cancer worldwide, as in US and other developed countries, the HCC cases are growing at extraordinary rate. Studies witnessed various dangers in HCC, including Hepatitis B/ C viral infection, exposure with some chemicals, high alcohol consumption, and metabolic diseases namely obesity and diabetes which rapidly rises in United State of America. Despite the etiologies below liver carcinogenesis that seemed to be comparatively beingwell outlined and therefore the actual pathways and mechanism leading to cancer development was

(Fig. 1: Metastasizing of cancer cells)
unclear even in today’s clinical technology. Chronic Hepatic problems, leading to inflammation and irregular regeneration of liver has advised as a significant phase in hepatocarcinogenesis. Carcinoma is one amongst prevailing styles of cancer globally (Alvare FA et al., 2013).

Mechanism of Hepatic Cancer
The mechanism underlying the inflammation caused because of growth promotion may well be greatly connected with liver injury followed with resultant irregular degeneration of hepatocytes. The induced hepatocyte proliferation throughout irregular hepatic regeneration that favors choice over remodeled cells and promotes induction of cellular growth. Development of HCC is regarded as very advanced and its occurrence can be attributed as multistep biological processes for malignant transformation over normal hepatocytes within varied factors, and due to genetic/epigenetic alterations, regulating aerobic stress, inflammation, and immunity area over the unit concerned. Till date, there are various studies that described molecular pathological approaches on HCC; however with imparting precise molecular mechanisms for HCC development remains to exist unclear.

Plant of Melastoma Malabathricum
Melastoma malabathricum belongs to the Melastomataceae family. It is also called the Singapore Rhododendron orSendudok. It is a erect shrub or small tree 1.5 to 5m tall. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, and infection during confinement, toothache, flatulence, sore legs, and thrush and also it is used by the Jah hut people in Malaysia to cure diarrhea (Sunilson J.A.J et al., 2009. Biological activities such as anti-inflammatory and hepatoprotective activities were reported (Balamurugan K et al., 2012 and Nishanti A et al., 2012).

Diethylnitrosamine (DENA)
Nowdays, study involves in emphasizing on chemoprotective effect of Melastoma malabathricum against DENA induced hepatic and renal cancer. It’s polar for understanding DENA elicited hepatic and renal cancer. Cancer of the liver is considered because he most prominent malignancies on a worldwide aspect, that is particularly determined in Asia and Africa. From this hepatocarcinoma accounted 80%-90% of cancer related complication in liver and is ranked 4th in terms of prominent causes in terms of inducing cancer mortality (Harris CC and Sun T 1984). Cancer condition of liver which is otherwise referred hepatic cancer resultant due to cancer occurrence taking place in liver. Cancer condition which is metastasized from elsewhere to liver called hepatic metastasis, which is found to be one of the pertinent condition of cancer than occurs within liver. The symptoms to HCC could be witnessed as sign of lump/pain within right facet below its skeletal structure, abdominal swelling, yellowish coloration of skin, bruising (simple), weight loss followed by weakness felt.
In case of renal cancer, urinary organ cancer is also called renal cancer could be group of cancer that begins within the urinary organ. Symptoms might involve blood in water, a lump identified on abdominal region, or back pain. Weight loss, fever and fatigue might also occur. The complication will comprise in spread over lungs/brain, as most type’s urinary organ cancer is renal cell cancer (RCC), transformation cell cancer (TCC), & Wilms growth. DEN is found to induce renal cancer and also the main mechanism concerned behind this is often oxidative stress (Siddiqi A et al., 2018).

MATERIAL AND METHOD

Collection of Plant
The plant *Melastoma malabathricum* linn (MM) was acquired from Sam Higginbottom Institute of Agriculture, Technology and Science in December 2019. The leaves was dried under normal environmental condition and authenticated by G.P Sinha, Botanical survey of India central Regional center 10, Chatham lines, Prayagraj 211002. Identification test for the authenticity was conducted as per the taxonomic features and was directly compared with its herbarium specimens that are available in department.

Extraction of Plant Leaves
Sucessive solvent extraction method was used for the extraction of leaves. The solvent used for the extraction was selected on the basis of polarity.

Preparation of Plant Extraction (Kumar V et al., 2013).
The leaves were chopped to smaller pieces and were thoroughly washed, dried under room temperature. By using Wiley mill, dried leaves were powdered. Accurately 100 gm of powdered leaves was weighed and transferred in a Soxhlet apparatus and extraction was performed using ethanol. Further, the extracts were concentrated using rotary evaporator, which was used for preliminary phytochemical screening as well as for anticancer activity.

Phytochemical Screening (Dr. Kokate C.K. et al., 2015).

Alkaloid.
1. **Dragendroff’s Test**: In a test tube, 2-3ml of filtrate was mixed with the few drop of Dragendroff’s Reagent (Potassium bismuth Iodide Solution). A reddish brown precipitate was formed confirming the presence of alkaloid.
2. **Hager’s Test**: 2-3ml of filtrate was incorporated with few drops in Hager’s Reagent (Saturated solution of Picric acid). Yellow precipitate was formed which confirmed the presence of alkaloid.
3. **Mayer’s Test**: In 1-2ml of filtrate, few drop of Mayer’s Reagent (Potassium Mercuric Iodide Solution) were added. Presence of alkaloid was confirmed by cream color precipitate fromed.
4. **Wagner’s Test**: 1-2ml of filtrate was mixed with few drop of Wagner’s Reagent (Iodine Potassium + Iodide solution). Change in colour to reddish brown confirmed the presence of alkaloid.

Glycoside
1. **Keller-killani Test**: In a test tube, 2ml of extract was added, glacial acid and one drop of 5% fecl3 and H2SO4 was added in the same test tube. Reddish brown color formed at the junction of two liquid layers along with the bluish green upper layer appears confirms the presence of glycoside.

2. **Legal Test**: In 2ml of extract, 1ml of Pyridine and 1ml of sodium Nitroprusside was added. Pink or red color was formed showing the presence of glycoside.

3. **Baljet's Test**: In the 2ml of extract, 2ml of sodium picrate solution was added. Yellow to orange color formed confirms the presence of glycoside.

**Experimental Animals**

*In vivo* study was performed on Albino Wister rat (100-150gm) in the animal house of Shambhunath Institute of Pharmacy, Prayagraj with the prior approval from Institutional Animal Ethical Committee (IAEC) bearing approval number IAEC/010/10/19. From studies performed according to CPCSEA. Healthy adult Albino Wister rat (100-150gm) of either sex were brought from Saha-Enterprise Kolkata, West Bengal (Reg.No:- 1828/PO/Bt/S/15/CPCSEA). The animals were housed under standard condition as prescribed and had a proper approach to water and feed, with the exclusion of food deprivation during the period of blood sampling throughout the experiment.

**Experimental Protocol**

All rats were divided randomly into five groups of six animals in each group. Rats were treated with a cancer causing agent (DEN) and tested drug. Single intraperitoneal administration DEN 2mg/kg (after 1 week) of rats. Later, rats were treated in MM suspension through oral route continued for 6 weeks. After 6 weeks rats were sacrificed and liver was isolated.

1. **Group I**: Normal
2. **Group II**: Control+DENA
3. **Group III**: DENA+MM (50mg/kg)
4. **Group IV**: DENA+MM (100mg/kg)
5. **Group V**: DENA+MM (200mg/kg)

**Preparation of Diethylnitrosamine (DEN)**

As per the reported method, (Afzal M et al., 2013) DEN (200mg/kg) was prepared in Phosphate buffer solution 4.5 and administered intraperitoneally to animals (Khan R et al., 2015).

**Preparation of 1% Carboxymethyl Cellulose (CMC) Suspension (V. Kumar et al., 2016).**

1 gm of CMC was added in motor and was grounded to uniform consistency with pestle. With the help of measuring cylinder, distilled water was slowly incorporated in the mortar and trituration was continued to avoid lump formation until the suspension was obtained. The suspension was afterwards divided into three equal parts in measuring cylinder. Varying concentration of plant extract (50,100,200 mg) was incorpated in each respective suspension.

**Assessment of Hepatic Cancer Action (Anwar Firoz et al., 2018).**

At the 6th week, blood samples were collected *via* heart puncture and the separation of serum was determined for the estimation of liver marker enzyme. Haemoglobin (Hb), Total leucocyte (TLC), Red blood cell (RBC), Erythrocyte Sedimentation rate (ESR), Serum Glutamic Oxaloacetic Transaminase (SGOT), and Serum Glutamic Pyruvic Transaminase (SGPT) test was done from Anoop Labs Pvt.Ltd (Reg. No- 2293/1886) Prayagraj.
Preparation of 40% formalin solution
40% if formalin solution was prepared further, rats were transferred in the dessicator containing chloroform, when rats were unconscious, they were sacrificed by using Tail Pulling / Cervical dislocation method.

Histopathological Examination
For the histopathological examination, rats were sacrificed from chloroform in a sealed dessicator. Their liver tissues appeared to immediately been removed and then preserved under 40% formalin solution. The 5 µm sample from hepatic tissues and were further then cut, deparaffinized, hydrated and then strained using hemotoxylin/ eosin. Prepared slide was then used to examine various changes in its occurrence in the liver and were compared with the other group.

Statistical analysis
From the data achieved, the resulted outcomes were achieved from one-way ANOVA as well as Dennett’s methods were carried out via Graph Pad Prism. The achieved value were exhibited from (p value <0.05) mean ± SEM. and regarded significant.

RESULT
Physicochemical Analysis of Melastoma Malabathricum Linn.
The physiochemical investigation of Melastoma Malabathricum Linn was carried out as per given Standard procedure. The leaves of Melastoma Malabathricum were extracted with ethanol solvent by successive Soxhlet apparatus. The yield of ethanol MM extract exhibited as 24.4% w/w.

Phytochemical Screening of Extract
The preliminary phytochemical screening of Melastoma Malabathricum Linn extract was performed as per the given procedure and the result obtained are mentioned in table 4.1

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Constituents</th>
<th>Tests</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Dragendorff's test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hagers Test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayers Test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagners Test</td>
<td>+ve</td>
</tr>
<tr>
<td>2.</td>
<td>Glycosides</td>
<td>Killer killani Test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Legal Test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baljet Test</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Note: ‘+’ sign indicated present and ‘−’ sign indicated absent
Phytochemical screening from ethanolic extract of *Melastoma Malabathricum* linn showed the Alkaloid and glycoside positive.

**Hepatic Cancer Activity**

The result of Hepatic cancer of extract leaves of *Melastoma Malabathricum* on DEN Induced Hepatic toxicity in rat. The liver marker enzyme, Hb, TLC, ESR, SGOT, SGPT blood serum and EDTA significantly rise level in DENA toxic group treated animals while comparing with normal/control group. The extract of leaves Melastoma Malabathricum at difference concentration low and high dose significantly reversed the level of liver marker enzyme represent while comparing with DENA toxic group treated Melastoma Malabathricum linn suspension.

**Table 2: Effect of Melastoma malabathricum Linn (MM) on blood serum (Hb, RBC, TLC, ESR, SGOT and SGPT) on DENA induced hepatic cancer.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Hb (Hb g/dl)</th>
<th>RBC (mil.Cu.mm. )</th>
<th>TLC (cells/cu.mm )</th>
<th>ESR (mm in 1 Hr)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal group</td>
<td>12.57 ± 12.50</td>
<td>7.59 ± 7.54</td>
<td>3850 ± 3847</td>
<td>0.84 ± 0.86</td>
<td>354.82 ± 354.80</td>
<td>136.29 ± 136.27</td>
</tr>
<tr>
<td>2.</td>
<td>Disease group</td>
<td>5.37 ± 5.34</td>
<td>2.53 ± 2.56</td>
<td>1640 ± 1637</td>
<td>0.3 ± 0.1</td>
<td>24.25 ± 24.20</td>
<td>36.35 ± 36.33</td>
</tr>
<tr>
<td>3.</td>
<td>DENA + Plant Extract MM.Linn (50mg)</td>
<td>7.39 ± 7.31</td>
<td>4.71 ± 4.68</td>
<td>1892 ± 1875</td>
<td>0.5 ± 0.4</td>
<td>40.84 ± 40.82</td>
<td>75.3 ± 74.2</td>
</tr>
<tr>
<td>4.</td>
<td>DENA + Plant Extract MM.Linn (100mg)</td>
<td>9.62 ± 9.59</td>
<td>6.14 ± 6.10</td>
<td>2133.94 ± 2133.90</td>
<td>0.45 ± 0.40</td>
<td>88.37 ± 88.34</td>
<td>107.92 ± 107.90</td>
</tr>
<tr>
<td>5.</td>
<td>DENA + Plant Extract MM.Linn (200 mg)</td>
<td>10.84 ± 10.79</td>
<td>6.52 ± 6.47</td>
<td>3083.34 ± 3083.30</td>
<td>0.67 ± 0.63</td>
<td>306.64 ± 306.60</td>
<td>110.24 ± 110.20</td>
</tr>
</tbody>
</table>

All values are expressed as means ± S.E.M, n = 5 groups; p value < 0.05, when compared with control group (ANOVA) followed by Dunnet’s t-test

(Fig.5: Effect of stress DENA & ethanol extract of Melastoma malabathricum Linn on hemoglobin (Hb) in stress induced hepatic cancer)
(Fig. 6: Effect of stress DENA & ethanol extract of Melastoma malabathricum Linn on total leucocyte count (TLC) in stress induced hepatic cancer)

(Fig. 7: Effect of stress DENA & ethanol extract of Melastoma malabathricum Linn on red blood cell count (TLC) in stress induced hepatic cancer)
(Fig. 8: Effect of stress DENA & ethanol extract of Melastoma malabathricum Linn on erythrocyte sedimentation rate (ESR) in stress induced hepatic cancer)

(Fig. 9: Effect of stress DENA & ethanol extract of Melastoma malabathricum Linn serum glutamic oxaloacetic transaminase (SGOT) in stress induced hepatic cancer)
Histopathological study
In the present study, the assessment of histopathology acquired from normal rat liver, represent the normal functioning and structural attributes identified in hepatic parenchyma, with no proof of damage in hepatocyte or dysplasia/ malignancy/ fibrosis. In DEN groups, the study showcased necrosis, with inflammatory infiltrate, and veins (central) appeared to be enclosed with extensive necrosis, followed by presence of clusters from hepatocyte necrosis as well as in nodules on trabeculae identified in malignant conditions of cells. From results of disease controlled groups, study indicated tumors with hepatocytes presence followed by effects of pleomorphism showed in 2-8 cell wide trabeculae separated using sinusoidal spaces (endothelial lined). Rat’s liver were treated via DEN and plant extract using CMC suspension combination, which showed ballooning degeneration which were noted in the case of hepatocytes and alongside with changes (reparative) noted among few hepatocytes with low index necrosis exhibiting unremarkable effect on central vein.
phytopharmacological screening of these plants for preparations and extract used to treat with other plant materials of MM were procured from Sam present study on the basis of their use in Ayurveda & presently. These cancer types appeared to have pharmaceutical value in the treatment of several ailments such as hypoglycemia and arthritic disorder that these plants were used in different herbal traditional medicine. The literature survey revealed that numerous diseases that have created concern across the globe, cancer remains to dominate the world population posing as a major class of disease that could be primarily characterized as uncontrollable cell growth, and was identified that almost 100 different forms of cancer types are classified presently. These cancer types appeared to have basically differentiated on the basis of its origin and type of cell. The study investigated the anti-cancer efficacy of Melastoma Malabathricum via determining the Chemoprotective effect of Melastoma malabathricum Linn (MM) against Diethyl nitrosamine (DENA) induced cancer. The plant species is particularly selected for the present study on the basis of their use in Ayurveda & traditional medicine. The literature survey revealed that these plants were used in different herbal preparations and extract used to treat with other ailments such as hypoglycemia and arthritic disorder lack scientific experimental evidence of their anticancer activity. In view of the above facts, it was thought worthwhile for taking the phytopharmacological screening of these plants for their anticancer activity in the case of DENA induced cancer cells using different histochemical studies. The plant materials of MM were procured from Sam Higginbottom Institute of Agriculture, Technology and Science in December 2019. The leaves was dried under normal environmental condition and authenticated by G.P Sinha, Botanical survey of India central Regional center. Extractions of leaves were done by successive slovent extraction. The solvent used for the extraction was selected on the base of polarity. Furthermore, the concentrated forms of ethanolic leaf extracts were used for preliminary phytochemical screeing and anticancer activity. From the phytochemical assessment involving several test indicated the presence of desired alkaloids and glycosides from the resultant outcomes. The study was further subjected to the histopathological examination. Initially, the experimental animals involving healthy adulty animals of stains of Albino Wister rat (100-150gm) of either sex were brought from Saha-Enterprise Kolkata, West Bengal (Reg.No:-1828/PO/Bt/S/15/CPCSEA). Animals were housed individually in Polypropylene cages, in a standrad environment codition, they were feed with standard rat pellet diet. The rats who acclimatized and 1 week prior to experimental studies. From experimental studies that conducted as per protocol of CPCSEA. The result of Hepatic cancer of extract leaves of Melastoma Malabathricum on DENA Induced Hepatic toxicity in rat was carried out with liver marker enzyme, Hb, TLC, ESR, SGOT, SGPT blood serum and EDTA significantly exhibiting increased level of DENA toxicity among the group treated with DENA compared to normal/control group. The extract of leaves Melastoma Malabathricum at different concentration ranges of low and high dose significantly reversed the level of liver marker enzyme among DENA toxic group when treated with Melastoma Malabathricum linn suspension.

**CONCLUSION**

From the detailed investigation on the present study inferred so far pertaining to MM effect on hepatoprotective activity from the ethanolic extracts of Melastoma Malabathricum Linn. Thus from the inferred study presented, it represented hepatoprotective activities showcased by extract of ethanolic Melastoma Malabathricum Linn. Leaves of DENA-induced hepatotoxicity showed significant improvement in liver enzymes level. The mechanism underlying with the bioactivity needs further investigation, but possibly due to antioxidant efficacy of investigated phytochemical attributes in forms of flavonoids endowed with radical scavenging attributes in leaves. This plant exhibiting immense potential as a new drugs. As the plant extracts of endowed with natural product that are applied for their pharmaceutical value in the treatment of several ailments. The extract of leaves Melastoma Malabathricum at 100mg/kg, (E) DENA + Plant extract Melastoma malabathricum Linn. (200mg/kg): exhibiting ballooning degeneration. (**Fig 11:** Histological images (mag 40X) showcasing various groups achieved from protocol: - (A) Normal: showed hepatic parenchyma (normal development), (B) indicating DEN: A: Spread of hepatocytes on vascular channels, as nodule sl under variable size, B: Lesion produced hepatocytes which are arranged on cords with giant hepatocyte, C: Secretion (Bile) in dilated canaliculi that are surrounded with cancerous cells, D: Microtrabecular forms of growth patterns, with tumor cells cords distanced due to sinusoidal voids, (C) DENA + Plant extract Melastoma malabathricum Linn. (50mg/kg), (D) DENA + Plant extract Melastoma malabathricum Linn. (100mg/kg), (E) DENA + Plant extract Melastoma malabathricum Linn. (200mg/kg): exhibiting ballooning degeneration.)
attributes. MM can be safely employed for long duration and also regarded cheap source endowed with active therapeutics that alleviates several generally occurring ailments due to poor and underprivileged Indians. Also, findings from research confirmed rationale over folkoric application on EN in cancer treatment and associated hepatic disorders. Furthermore studies are under progress on evaluating their molecular mechanism governing with their hepatoprotective agents and would suggest plausible clinical effect and application of EN extracts.

AUTHOR CONTRIBUTION
Mr. Kuldeep Singh was HOD of Pharmacology department of Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj and my project guide also. Singh sir gave a strong platform to perform this project. With the help of Singh sir my college provided statistical support in analyzing data for instrumentation analysis. My colleague Nikhil Kushwaha helped me in every step of unit process and participated in the sequence alignment and drafted the manuscript with me. I am Vipin Kesharwani carried out all work related to this project with the help of my guide. And prepare all data that present in this manuscript and also design a sequence alignment and drafted in this manuscript. All author approved that they final manuscript.

CONFLICT OF INTEREST
The author declares that they have no conflict of interest.

REFERENCES

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Conflict of Interest: None declared

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