Research Article

Anti-Inflammatory Activity Of Momordica Charantia

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ABSTRACT

Background:
Momordica charantia, commonly known as bitter melon, is a medicinal plant traditionally used for its various health benefits, including its potential anti-inflammatory properties. This study was aimed to evaluate the anti-inflammatory activity of Momordica charantia extracts scientifically.

Methods:
The research involved the extraction of bioactive compounds from the leaves and fruits of Momordica charantia using different solvents (ethanol, methanol, and aqueous). The anti-inflammatory activity was assessed through in vitro assays, including the inhibition of nitric oxide production in lipopolysaccharide (LPS)--stimulated macrophages, and in vivo models, such as carrageenan-induced paw oedema in rats. Additionally, the phytochemical constituents of the extracts were analysed using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS).

Results:
The study found that the ethanol and methanol extracts of Momordica charantia exhibited significant anti-inflammatory activity in both in vitro and in vivo models. The inhibition of nitric oxide production in LPS-stimulated macrophages was observed, with the ethanol extract showing the highest inhibition rate. In the carrageenan-induced paw oedema model, the ethanol extract demonstrated a significant reduction in paw swelling compared to the control group. Phytochemical analysis revealed the presence of key bioactive compounds, including flavonoids, phenolic acids, and triterpenoids, which are likely responsible for the observed anti-inflammatory effects.

Conclusion:
Momordica charantia exhibits promising anti-inflammatory activity, which is attributed to its rich phytochemical composition. These findings support the traditional use of Momordica charantia in inflammatory conditions and suggest its potential as a natural therapeutic agent for managing inflammation. Further studies are warranted to isolate specific active compounds and explore their mechanisms of action.

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INTRODUCTION

Inflammation is a complex biological response that serves as a protective mechanism against infections, injuries, and harmful stimuli. While acute inflammation is essential for healing, chronic inflammation can lead to various pathological conditions, including arthritis, cardiovascular diseases, diabetes, and cancer. Traditional anti-inflammatory treatments, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are effective but often associated with significant adverse effects, especially when used long-term. This necessitates the search for safer, natural anti-inflammatory agents. Momordica charantia, commonly known as bitter melon or bitter gourd, is a climbing vine of the Cucurbitaceae family, prevalent in tropical and subtropical regions. It has been extensively used in traditional medicine systems across Asia, Africa, and the Caribbean for its therapeutic benefits. Historically, Momordica charantia has been used to manage conditions such as diabetes, gastrointestinal issues, infections, and immune disorders. Modern scientific investigations have corroborated some of these traditional uses, attributing various pharmacological properties to the plant, including anti-diabetic, antioxidant, anticancer, and anti-inflammatory activities. The anti-inflammatory properties of Momordica charantia are particularly noteworthy given the current interest in natural and alternative medicine.

MATERIALS AND METHODS

Collection and authentication of plant

The project work was carried out in the Adina Institute of Pharmaceutical Sciences, Sagar. Momordica charantia was procured from the local market in Sagar. The vegetable was authenticated in botany department of Dr. Hari Singh Gour university, Sagar, M.P. The vegetable was washed and cleaned before being cut into pieces. For one week, the pieces were dried at room temperature in a shaded area. After that, dried pieces were ground and the powder was collected as a sample, homogenized fresh wild bitter gourd (whole fruits including seeds) into a pulp.

Phytochemical analysis

Preliminary phytochemical screening of Momordica charantia was carried out to determine the existence of plant secondary metabolites (qualitatively) using the standard methods.

Assessment of flavonoids and terpenoids

About 1 ml of the sample was dissolved in 1 M of HCl (5 ml each) and diluted NaOH. The existence of flavonoids was expressed when a yellow solution became colorless. Similarly, the sample (1 ml) was thoroughly mixed with chloroform (2 ml) and concentrated sulfuric acid (3 ml) to form a layer. The appearance of reddish brown indicates the presence of terpenoids.

Assessment of Alkaloids

200 mg of the sample was warmed up in a boiling water bath with 5 mL of 2N HCl. The chilled mixture was then purified and divided into two equal parts. One part was treated with a few drops of Mayer’s reagent and the other with...
Dragendorff’s reagent. The existence of alkaloids was expressed by the turbidity of the resulting precipitates.52

Assessment of Saponins
About 200 mg of the sample was shaken with 5 ml of distilled water in a test tube and heated in a water bath to boil. The existence of saponins was expressed by the formation of strong and stable foam.53

Estimation of total phenolic content
In this study, I evaluated the total phenolic content in an aqueous extract of Momordica charantia (5g/50 ml) using a spectrophotometric method. Briefly, Folin-Ciocalteu’s reagent (0.5 ml) and aqueous extract (1 ml with 10 mL of distilled water) were mixed and incubated for 3 min. Following that, 1 mL of saturated Na2CO3 was added to the solution, increasing its volume to 25 mL. Finally, samples of Momordica charantia were laid down in a dark place for 1 h, and the absorbance was measured at 750 nm. Aqueous extract samples of Momordica charantia were analyzed in triplicates, and its mean value of absorbance was taken. Gallic acid (5, 10,20, 40, 60, 80, 100, and 150 mM) was used as the standard in this study, and it was taken at various concentrations to generate a calibration curve, after which the results were expressed in mM.54

Animal Treatment and Grouping
Our 72 six-week-old male BALB/c mice (body weight approximately 16–19 g) were purchased from the Sagar. They were first fed a rodent chow diet for one week of adaptation. When their body weights averaged approximately 22 g, I randomly divided them based on their body weights. Mice were fed for a total of four weeks during the experiment. Feed intake levels during the experimentation period were recorded daily, body weight was recorded weekly. Each mouse was fed a 4 g/day test feed. An excess supply of drinking water was used to allow free intake. This study used the endotoxin model to induce acute septicemia reactions. Before mice sacrifice, other than the normal (N) group, each group received intraperitoneal injections of lipopolysaccharide (LPS). The LPS-induced sepsis mice were made by injecting 15 mg/kg BW of LPS (E. coli O127: B8; Sigma, St. Louis, MO, USA) in 0.4 mL normal saline intraperitoneally (i.p.). The positive control (P) group received intravenous injections of the anti-inflammatory drug pyrrolidinedithiocarbamic acid ammonium salt (PDTC) 1 h before the LPS injection. The normal control group was injected with normal saline.55

Six experimental groups were used, each identified by the names and symbols indicated below:
1. N: Normal group (i.p. normal saline), fed the chow diet.
2. S: Sepsis group (i.p. LPS, 15 mg/kg BW), fed the chow diet.
3. L: Sepsis group with low-dose (1%) wild bitter gourd lyophilized powder added, fed feed including 1% wild bitter gourd lyophilized powder.
4. M: Sepsis group with moderate-dose (2%) wild bitter gourd lyophilized powder added, fed feed including 2% wild bitter gourd lyophilized powder.
5. H: Sepsis group with high-dose (10%) wild bitter gourd lyophilized powder added, fed feed including 10% wild bitter gourd lyophilized powder.
feed including 10% wild bitter gourd lyophilized powder.

6. P: Positive control group (i.p. PDTC, 50 mg/kg BW), fed the chow diet.

Heat-Induced Hemolysis

In this experiment, whole blood cell suspension (50 µl) containing a similar volume of different concentrations of Momordica charantia was dissolved in PBS (2.9 ml). Incubate the samples for 20-30 minutes at 54°C in a shaking water bath. After incubation, centrifuged these samples at high speed, collected the supernatant, and measured its OD value at 570 nm using a UV-visible spectrophotometer. In this experiment, PBS served as the negative control for these studies. The level of hemolysis in whole blood cell suspension using Momordica charantia was calculated based on this equation, as mentioned below:

\[
\text{% inhibition of hemolysis} = 100 \times \left(1 - \frac{A_2}{A_1}\right),
\]

where

\(A_1\) = control absorbance, and

\(A_2\) = Momordica charantia absorbance value.

Proteinase Inhibitory Activity

In this experiment, we prepared a reaction mixture (2 ml) consisting of trypsin (0.06 mg), Tris-HCl buffer (20 mM, pH 7.4) and added different concentrations of Momordica charantia. Incubate the samples at 37°C for 10 min and then add casein (1 ml, 0.8%). Again, incubate the samples for 30 min, and then perchloric acid (70%; 2 ml) was added to stop its reaction rate. Centrifuging these sample mixtures at high speed determined their absorbance value in the supernatant and measured them at 210 nm. In this experiment, PBS served as the control for these studies. In this assay, we determined its proteinase inhibitory activity based on this equation as mentioned below:

\[
\text{% proteinase inhibitory activity} = 100 \times \left(1 - \frac{A_2}{A_1}\right),
\]

where

\(A_1\) = control absorbance, and

\(A_2\) = Momordica charantia absorbance value.

In vitro anti-inflammatory activity

To evaluate its denaturation of protein assay, an aqueous extract of Momordica charantia was applied against a specific protein antigen. Briefly, an aqueous extract of Momordica charantia using a variable concentration (0.5-500 µg/ml) was homogenized separately with typhoid vaccine (25 µg/ml; 1 ml; Bharat Biotech company) and bovine serum albumin (BSA, 5g/100 ml; 1 ml). Incubate these extracts of Momordica charantia along with BSA or typhoid vaccine at 37°C for 30 minutes whereas the control tube had a combination of distilled water and BSA or typhoid vaccine. For this experiment, denaturation of the proteins with or without extracts was caused by placing the samples of an aqueous extract of Momordica charantia in a water bath for 10 minutes at 70°C. The mixture was cooling inside the ambient room temperature, and the activity of each mixture was measured at 660 nm. Each test was done three times. The following formula was used to calculate the inhibition percentage:

\[
\text{% inhibition in protein} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100.
\]

Blood preparation and biochemical assay

After four weeks’ treatment, mice were fasted overnight (twelve hours) and decapitated. Blood was centrifuged at 1700 g at 4°C for 30 min to separate serum gauged by enzymatic methods, using commercial kits (RANDOX, Amtrim, UK) for TG, cholesterol, glucose and non-esterified fatty acid (NEFA), this biochemical analysis as previously described. Serum inflammatory
mediator included NO, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and C-reactive protein (CRP), with concentration analyzed by clinical test kits (Roche Cobas Mira plus, Germany).61

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Momordica charantia</th>
<th>Total phenolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.9</td>
<td>7.84 ± 0.22</td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
<td>11.2 ± 0.64</td>
</tr>
<tr>
<td>3</td>
<td>15.6</td>
<td>18.14 ± 0.78</td>
</tr>
<tr>
<td>4</td>
<td>31.25</td>
<td>26.6 ± 1.14</td>
</tr>
<tr>
<td>5</td>
<td>62.5</td>
<td>33.4 ± 1.98</td>
</tr>
<tr>
<td>6</td>
<td>125</td>
<td>43.6 ± 1.56</td>
</tr>
<tr>
<td>7</td>
<td>250</td>
<td>57.2 ± 2.12</td>
</tr>
<tr>
<td>8</td>
<td>500</td>
<td>66.4 ± 1.94</td>
</tr>
</tbody>
</table>

Readings were taken and the phenolic content through a calibration curve obtained from the standard (gallic acid) was linear with $y = 0.0343x + 0.0916; R^2 = 0.972$.

**In vitro anti-inflammatory activity**

An aqueous extract of Momordica charantia in human whole-blood cell suspension was shown to inhibit hemolysis in a concentration-dependent manner. To determine its in vitro anti-inflammatory activity. Similarly, anti-inflammatory activity was also estimated against BSA and typhoid vaccines using a variable concentration of the aqueous extract of Momordica charantia shown in Fig.2. The results showed that the aqueous extract at higher concentrations showed significant declines in protein denaturation with reference to BSA and typhoid vaccine as compared to the control. In addition, the proteinase inhibitory activity of Momordica charantia is shown in Fig. 3, and the inhibition levels were within the range of 21.2–45.4 % as compared to the control.63
Fig. 3. Proteinase inhibitory activity.

Effects of wild bitter gourd on body weight change and lipid metabolism
Table 2 shows changes in body weight and intake level for each group: body weights of Groups L, M, and H significantly lower than those of Groups N, S, and P. Group H had most significant weight-loss effect. Organ lesion caused by sepsis spawns’ metabolic abnormalities for blood lipids (TG, NEFA, and cholesterol) within the body. This is significantly reduced after adding wild bitter gourd, trending toward a dose response. Table 3 shows that the blood glucose abnormalities of Group S followed low blood glucose situations. This may be caused by multiple organ failure (MOF) following sepsis and is considered a complication thereof. Wild bitter gourd is known to activate PPARα, thus moderating lipid metabolism; its active components, such as conjugated linoleic acid (CLA), are speculated to activate PPARα, thereby facilitating β-oxidation for fatty acids and maintaining constancy of lipid metabolism within cells. PPARα also regulates ketogenesis within the body, thereby moderating metabolism and balance of lipids. Earlier studies confirm wild bitter gourd as containing PPARα and PPARγ activators 64 that facilitate lipid metabolism, reduce blood lipids, and cause anti-inflammatory activity 65. This subsequently reduces fat accumulation, resulting in a weight-loss effect. Besides reducing blood glucose concentrations, PPARγ activation reduces inflammation reactions.65 Molecular mechanisms may entail inhibition of cytokines secreted by monocytes following PPARγ activation66. This interferes with the transmission of messages for NF-kB, signal transducers and activators of transcription (STAT), activator protein-1 (AP-1), etc., inhibiting expression of inflammatory genes like IL-1, IL-2, IL-6, IL-8, TNF-α, and metalloproteinase (MMPs)67. PPAR agonists currently treat atherosclerosis; they activate both receptors simultaneously and are regarded as having the greatest potential. Whether the significant reduction in body weight of mice fed 10% wild bitter gourd wreaks negative physiological effects merits further research 67.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Mice</th>
<th>Initial BW (g)</th>
<th>Final BW (g)</th>
<th>Food Intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td>23</td>
<td>25</td>
<td>3.8</td>
</tr>
<tr>
<td>S</td>
<td>12</td>
<td>24</td>
<td>27</td>
<td>3.5</td>
</tr>
<tr>
<td>L</td>
<td>12</td>
<td>23</td>
<td>25</td>
<td>3.4</td>
</tr>
<tr>
<td>M</td>
<td>12</td>
<td>22</td>
<td>23</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Table 3 Serum triglyceride, cholesterol, NEFA, and glucose concentrations of LPS-induced sepsis mice.

<table>
<thead>
<tr>
<th>Group</th>
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<tbody>
<tr>
<td>Number of Mice</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
</tr>
<tr>
<td>N</td>
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<tr>
<td>S</td>
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<tr>
<td>L</td>
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<tr>
<td>M</td>
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<td>H</td>
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<td>P</td>
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</tbody>
</table>

Effects of wild bitter gourd on inflammation response and organ damage

Table 4 plots pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) concentrations in spleens of mice with sepsis as definitely higher than those in Group N. These ratios dropped sharply after wild bitter gourd was added to diets. Secretions of anti-inflammatory cytokine IL-10 increased, presenting dose-response trends. Serum inflammatory mediators like NO, AST, ALT, and C-RP, all fell significantly in mice after four weeks of test feeds containing various doses of wild bitter gourd, consequently reducing damage to organs and tissue. Group H had the most significant results, falling to values equal to those of Group N (Table 3). When organs are damaged, AST (GOT) and ALT (GPT) within cells are released into the serum. Extant literature indicates that after sepsis occurs, cell necrosis increases during the septic process, releasing more liver cell enzymes. AST and ALT concentrations in the blood rise 68, causing inflammation response accompanied by severe organ damage 69.

Table 4. Spleen level of inflammatory cytokines concentration of LPS – induced sepsis mice

<table>
<thead>
<tr>
<th>Group</th>
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<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>TNF-α (µg/mL)</td>
</tr>
<tr>
<td>IL-1β (µg/mL)</td>
</tr>
<tr>
<td>IL-6 (µg/mL)</td>
</tr>
<tr>
<td>IL-10 (µg/mL)</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>H</td>
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<tr>
<td>P</td>
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</table>

Table 5. Serum inflammatory mediator concentrations of LPS-induced sepsis mice

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<tr>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>NO (µM)</td>
</tr>
<tr>
<td>AST(GOT) (unit/L)</td>
</tr>
<tr>
<td>ALT(GPT) (unit/L)</td>
</tr>
<tr>
<td>C-RP (ng/mL)</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>S</td>
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<tr>
<td>L</td>
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<td>M</td>
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</table>

LPS is currently known to increase the transcriptional effects of iNOS mRNA by activating NF-κB. Two hours following acute attacks of sepsis, NO is produced excessively within the kidneys, resulting in low blood pressure and tachycardia. Extant literature indicates that activation of PPARγ can inhibit the formation of inflammatory mediators like TNF-α, IL-1β, and...
IL-6. PPARγ also reduces iNOS activity by inhibiting transcription factor NF-κB, thence alleviating inflammation. This study indicates 10% wild bitter gourd diet significantly inhibiting expression of iNOS protein, reducing formation of inflammatory mediator NO. Likewise, adding wild bitter gourd to diets can reduce formation of inflammatory mediator PGE2 by reducing expression of COX-2 protein. This reduces production of pro-inflammatory cytokines and other substances. Anti-inflammatory cytokine IL-10 also rises significantly, achieving anti-inflammatory effects. Extant literature indicates butanol-soluble fraction of its placenta extract strongly suppressing LPS-induced TNF-α yield in RAW264.7 cells. Anti-inflammatory components were identified as 1-α-linolenyl-lyosphosphatidylcholine (LPC), 2-α-linolenoyl-LPC, 1-linoleoyl-LPC, and 2-linoleoyl-LPC. In Groups L, M, and H, since wild bitter gourd contains substances capable of activating PPARγ, severity of septic inflammation reaction was retarded, thus reducing organ damage. Extant literature indicates liver vascular sinus accumulating formation of substantial amounts of platelets, red blood cells, white blood cells, and micro thrombosis during endotoxic shock. This causes expansion and obstruction to the vascular sinus, thereby damaging liver function. Acute septicemia causes substantial increases in vascular permeability and rapid heart rate. The ensuing insufficient return of blood to organs affects heart weight and blood volume. This study also indicates that, regarding weights of organs (liver, kidneys, and spleen), Group H proves able to reduce organomegaly (data not shown) compared with Group S. These will help design human studies necessary to prove efficacy of bitter gourd against clinical sepsis.

DISCUSSION:

LPS is currently known to increase transcriptional effects of iNOS mRNA by activating NF-κB. Two hours following acute attacks of sepsis, NO is produced excessively within kidneys, resulting in low blood pressure and tachycardia. Extant literature indicates that activation of PPARγ can inhibit formation of inflammatory mediators like TNF-α, IL-1 β, and IL-6 78. PPARγ also reduces iNOS activity by inhibiting transcription factor NF-κB, thence alleviating inflammation. This study indicates 10% wild bitter gourd diet significantly inhibiting expression of iNOS protein, reducing formation of inflammatory mediator NO. Likewise, adding wild bitter gourd to diets can reduce formation of inflammatory mediator PGE2 by reducing expression of COX-2 protein. This reduces production of pro-inflammatory cytokines and other substances. Anti-inflammatory cytokine IL-10 also rises. In literature, scientific-based studies related to medicinal plants were taken into consideration to understand their economic and medicinal importance. Some of the most familiar examples of medicinal plants are Azadirachta indica, Boswellia serrata, Withania sominiferra, Picrohiza kurroa, etc. So, these medicinal plant-based studies are well appreciated by authors all over the world, who understand their immunobiological activities. Because of this, our major objective is to evaluate the antimicrobial, antioxidant, and anti-inflammatory activity of Momordica charantia. Preliminary studies were carried out on Momordica charantia to identify the existence of secondary metabolites that are present in the aqueous extract. So, these studies revealed the existence of flavonoids, terpenoids, alkaloids, and saponins in Momordica charantia. In addition, I also estimate the phenolic content which is one of the most common anti-inflammatory compounds found in plants. In light of this, our primary focus is on phenolic content (an important indicator of anti-inflammatory potential) and correlates phenolic content data with Momordica charantia an anti-inflammatory activity. Our
results may find that higher phenolic content is reported at higher concentrations using gallic acid as the standard. In other words, Momordica charantia regarded them as one of the richest sources of phenolic compounds, and they were widely used in traditional medical systems. In the literature, protein denaturation is reported only due to inflammatory processes like arthritis. The major role of NSAIDs is to protect against denaturation of protein molecules. In other words, a decline in the rate of protein denaturation may play a very crucial role, especially seen in the antirheumatic activity of NSAIDs. Various studies were conducted concerning plant components and protein denaturation. In short, protein denaturation-based studies were applied to understand the anti-inflammatory action of Momordica charantia. In literature, inflammation is directly associated with protein denaturation and the results from these studies showed that Momordica charantia significantly inhibited the protein at higher doses as compared to the control. In other words, Momordica charantia had the highest anti-inflammatory potential (strong inhibition of protein denaturation) in the case of the typhoid vaccine as compared to BSA. In addition, proteinases are directly involved or associated with arthritic reactions because leukocytes containing proteinases may directly impact the development of tissue damage during inflammatory processes. Protease inhibitors have also been shown in the literature to provide significant protection against infectious diseases. Therefore, the existence of molecules present in Momordica charantia may directly contribute to its anti-inflammatory activity. In short, Momordica charantia in the form of an aqueous extract has anti-inflammatory potential.

CONCLUSION:
Maximum anti-inflammatory activities were observed in an aqueous extract of Momordica charantia, which showed strong positive correlations with phenolic content. The results from this study revealed that Momordica charantia contains a substantial phenolic content, which was suggested to be the major contributor to its antioxidant, antibacterial, and anti-inflammatory activities. Future research work will be focused on the active metabolite of Momordica charantia to discover effective pharmacological agents.

CONFLICTS OF INTEREST-
None

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