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Biogenic Nanoparticle Synthesis Using Ganoderma Macrofungi and Its Biomedical and Environmental Applications

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ABSTRACT

Ganoderma is a notable Indian conventional mushroom utilized in different ethnomedicinal practices. New advancements connected with this mushroom and their exercises are consistently reported. A few researchers working with spores of this therapeutic mushroom however data of this species are still limited. This review describes about the main advances for the extraction of biocomponents and their pharmacological and cell protective effects on health system because of their importance in drug discovery. Recognizing the bioavailability of the naturally dynamic parts of G. lucidum after oral administration, and very little has been published in this area. Although single compounds can mediate a biological effect, nutraceuticals often act through the synergistic activity of multiple metabolites. It is significant to gain a better understanding of the bioavailability of the active components in this mushroom under various conditions to ensure robust study designs and to maximise consumer advantage. Nanoparticles derived from macro fungi, including various mushroom species like Ganoderma spp. are well known to possess immune-modulatory, high nutritional, antimicrobial, antioxidant, and anticancerous effect. Fungi have intracellular metal uptake capacity and supreme wall binding ability. In this review also discussed the nanoparticles synthesis from micro fungi of Ganoderma species and mechanism of nanoparticles derived from the micro fungus.

Keywords: mushroom, biomedical, compounds, peptidoglycan, immunomodulation

INTRODUCTION

Ganoderma lucidum has an ornamental fungus use for longevity and promoting health in Japan, China and other Asian countries[1]. It looks different dark glossy exterior and a woody surface. The Latin word *lucidus* means brilliant and denotes to the smooth appearance of the surface of the mushroom. In north India, *G. lucidum* is termed lingzhi, In Japan the name is reishi. *G. lucidum* is unique in its pharmacological and nutritional value[2]. A variety of commercial *G. lucidum* products are accessible in various forms like powders, dietary supplements and tea. These are produced from various portion of the mushroom, including spores, fruit body and mycelia. *G. lucidum* has been recognized as a medicinal mushroom for over 2000 years. Its powerful effects have been documented in ancient scripts. The family Ganodermataceae designates basidiomycetous polypore fungi with a double-walled basidiospore[3,4]. Basidiocarps of this genus have a smooth surface that is related with the occurrence of thick enclosed pilocystidia fixed in an extracellular melanin medium. The morphological appearances are focus to variation resulting from modifications in cultivation with various geographical areas under diverse climatic circumstances and the natural genetic evolution like recombination mutation of individual species. Consequently, the use of macroscopic characteristics has resulted in a large number of synonyms and a confused, overlapping, and unclear taxonomy for this mushroom. Some taxonomists also consider macromorphological features to be of limited value in the identification of *Ganoderma* species due to its high phenotypic plasticity[5]. Molecular-based methodologies adopted for identifying *Ganoderma* species include recombinant (rDNA) sequencing, random amplified polymorphic DNA-PCR (RAPD; PCR stands for polymerase chain reaction), internal transcribed spacer (ITS) sequences. Different members of the *Ganoderma* genus need different conditions for growth and cultivation. Artificial cultivation of *G.*

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lucidum has been achieved using substrates such as grain, sawdust, wood logs and cork residues[6–8]. Nowadays, mushrooms show significant potential in metal nanoparticle (NP) synthesis and multifaceted applications. Many reports on mycogenesis derived from nanoparticles have been reported. But the mechanisms of synthesis of nanomaterials of mycogenic with different size and topologies are not well understood. Fungus groups consists of molds, yeast, rust and mildew. The assistances and appropriate use of fungal cells NP are attributed to the release of more extracellular enzymes that can serve as bio-reducing and stabilizing agents for NP synthesis[9,10]. Moreover, fungal-derived NPs are much better than the bacteria-derived NPs.

Bioactive compounds available in Ganoderma

Most mushrooms are composed of around 90% water by weight. The remaining 10% consists of 10–40% protein, 2–8% fat, 3–28% carbohydrate, 3–32% fiber, 8–10% ash, and some vitamins and minerals, with potassium, calcium, phosphorus, magnesium, selenium, iron, zinc, and copper accounting for most of the mineral content. In a study of the nonvolatile components of *G. lucidum*, it was found that the mushroom contains 1.8% ash, 26–28% carbohydrate, 3–5% crude fat, 59% crude fiber, and 7–8% crude protein[11,12].

Peptidoglycans and polysaccharides

Fungi are remarkable for the variety of high-molecular-weight polysaccharide structures that they produce, and bioactive polyglycans are found in all parts of the mushroom[13]. Polysaccharides represent structurally diverse biological macromolecules with wide-ranging physiochemical properties. Various polysaccharides have been extracted from the fruit body, spores, and mycelia of lingzhi; they are produced by fungal mycelia cultured in fermenters and can differ in their sugar and peptide compositions and molecular weight (e.g., ganoderans A, B, and C). *G. lucidum* polysaccharides (GL-PSs) are reported to exhibit a broad range of bioactivities, including anti-inflammatory, hypoglycemic, antiulcer, antitumorigenic, and immunostimulating effects[14,15]. Polysaccharides are normally obtained from the mushroom by extraction with hot water followed by precipitation with ethanol or methanol, but they can also be extracted with water and alkali. Structural analyses of GL-PSs indicate that glucose is their major sugar component[16–18]. GL-PSs are heteropolymers and can also contain xylose, mannose, galactose, and fucose in different conformations, including 1–3, 1–4, and 1–6-linked β and α-D (or L)-substitutions. Branching conformation and solubility characteristics are said to affect the antitumorigenic properties of these polysaccharides[19]. The mushroom also consists of a matrix of the polysaccharide chitin, which is largely indigestible by the human body and is partly responsible for the physical hardness of the mushroom. Numerous refined polysaccharide preparations extracted from *G. lucidum* are now marketed as over-the-counter treatment for chronic diseases, including cancer and liver disease[20–22].

Triterpenes

Triterpenes are a subclass of terpenes and have a basic skeleton of C_{30}. In *G. lucidum*, the chemical structure of the triterpenes is based on lanostane, which is a metabolite of lanosterol, the biosynthesis of which is based on cyclization of squalene[23] . Extraction of triterpenes is usually done by means of methanol, ethanol, acetone, chloroform, ether, or a mixture of these solvents. The extracts can be further purified by various separation methods, including normal and reverse-phase HPLC[24,25]. Elemental analysis of log-cultivated fruit bodies of *G. lucidum* revealed phosphorus, silica, sulfur, potassium, calcium, and magnesium to be their main mineral components. Iron, sodium, zinc, copper, manganese, and strontium were also detected in lower amounts, as were the heavy metals lead, cadmium, and mercury. Freeze-dried fruit bodies of unidentified *Ganoderma* spp[26]. collected from the wild were reported to have a mineral content of 10.2%, with potassium, calcium, and magnesium as the major components. Lectins were also isolated from the fruit body and mycelium of the mushroom[27,28].

Pharmacological application

G. lucidum has been used for hundreds of years as a health promotion and treatment strategy. there are now

many published studies that are based on animal and cellculture models and on in vitro assessment of the health effects of *G. lucidum*. there are also some reports of human trials in the field. *G. lucidum* is a popular supplement

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue III, March 2026

taken by healthy individual to boost the immune system and by cancer patients along with conventional therapies[29]. Many polysaccharides and triterpenes, the two major groups of components in the mushroom, exhibit chemopreventive and/or tumoricidal effects, as proved by numerous studies from in vitro experiments and animal and human in vivo studies. hrough the regulation of expression of different signals, tumor cells were arrested by *G. lucidum* at different points of cell cycle, for example, breast at G0/G1 phase; lung at G1 phase; liver at G1/G2 phase; and bladder, prostate, and leukemia at G2 phase[30]. A selenium-enriched extract of *G. lucidum* mycelia was shown to induce G1/S phase arrest in human erythroid chronic myeloid leukemia K562 cells. The potential antiangiogenic activities of *G. lucidum* have been demonstrated in ex vivo chick embryo chorioallantoic membrane (CAM) assay[30,31]. Polysaccharide peptide and ethanol extract from *G. lucidum* has been proved to decrease microvessels around a microfiber filter disc containing an embryo with intact yolks. Using a prostate cancer cell line, two angiogenic factors, known as vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1, were suppressed by *G. lucidum* through inhibition of the ras/extracellular signal–regulated kinase (Erk1/2) and Akt signaling pathways[32,33]. *G. lucidum* is a major component of many traditional botanical formulations, such as TBS-101, which was demonstrated to inhibit tumor growth and invasion in PC-3-implanted mice[34,35]. Oral administration of triterpenoid fractions for 18 consecutive days inhibited Martigel-induced angiogenesis, which significantly reduced tumor weight and the number of tumor cell colonies that had metastasized to the liver in female C57BL/6J strain mice with intrasplenic implantation of Lewis lung cancer cells. An additive effect was seen when *G. lucidum* was given in combination with cytotoxic antineoplastic drugs, and there was a suggestion of a possible synergistic effect with cisplatin[36,37]. The chemopreventive activities of the mushroom on prostate cancer were demonstrated by a triterpenoid-rich extract of *G. lucidum* that suppressed the ventral prostate growth induced by testosterone[38].

Immunomodulation effect

There is considerable evidence to support the immunostimulating activities of *G. lucidum* via induction of cytokines and enhancement of immunological effector. Different components from *G. lucidum* were proved to enhance the proliferation and maturation of T and B lymphocytes, splenic mononuclear cells, NK cells, and dendritic cells in culture in vitro and in animal studies in vivo[39,40]. It was reported also that TNF-α and IL-6 production were stimulated in human and murine macrophages by *G. lucidum* mycelia. The cytotoxicity of CIK cells was correlated well with the expression of perforin and granzyme B induced by IL-2 and anti-CD3. Results indicated that GL-PSs enhance IL-2 and TNF-α production as well as protein and messenger ribonucleic acid (mRNA) expression of granzyme B and perforin in CIK cells culture[41].

Antioxidant activity

Antioxidants protect cellular components from oxidative damage, which is likely to decrease risk of mutations and carcinogenesis and also protect immune cells, allowing them to maintain immune surveillance and response. Various components of *G. lucidum*, in particular polysaccharides and triterpenoids, show antioxidant activity in vitro[42]. The protective effects of *G. lucidum* on DNA strand scission induced by a metal-catalyzed Fenton reaction, ultraviolet irradiation, and hydroxyl radical attack were shown in agarose gel electrophoresis in vitro. Two antioxidant-enriched extracts from *G. lucidum* acted oppositely in premalignant HUC-PC cells under carcinogenic attack[43]. The results suggested that different effects of *G. lucidum* could be exhibited by different extractable components in bladder chemoprevention. Methanol extracts of *G. lucidum* were reported to prevent kidney damage (induced by the anticancer drug cisplatin) through restoration of the renal antioxidant defense system[44].

Antiviral and Antibacterial activity

Isolation of various water- and methanol-soluble, high-molecular-weight PBPs from *G. lucidum* showed inhibitory effects on herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and vesicular stomatitis virus (VSV) New Jersey strain in a tissue culture system[45,46]. Using the plaque reduction method, a significant inhibitory effect was seen at doses that showed no cytotoxicity. he cells were treated before, during, and after infection, and viral titer in the supernatant of cell culture 48 hours postinfection was determined[47,48]. A dried hot water extract of *G. lucidum* taken orally (equivalent to 36 or 72 g of dried mushroom per day) was used as the sole treatment for postherpetic (varicella zoster virus) neuralgia in 4 elderly

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue III, March 2026

patients. This treatment was reported to dramatically decrease pain and promote the healing of lesions, without any toxicity even at very high doses[49].

Antidiabetic effect

The administration of ganoderans A and B, two polysaccharides isolated from fruit-body water extracts, by i.p. injection to normal and alloxan-induced diabetic mice significantly decreased (by up to 50%) the plasma glucose concentrations, and the hypoglycemic effect was still evident after 24 hours. Using a mouse model, ganoderan B was also reported to increase plasma insulin, decrease hepatic glycogen content, and modulate the activity of glucose-metabolizing enzymes in the liver[50]. Polysaccharides extracted from *G. lucidum* and given orally to rats for 28 days were found to ameliorate cirrhosis induced by biliary ligation. The treatment significantly decreased ligation-induced increases in serum biochemical markers of liver damage[51,52].

Bioactivities on nanoparticles

solution of AgNO3 was used as a precursor with Vietnamese G. lucidum extract for the synthesis of colloidal AgNPs. Nanoscalecarriers offer several advantages including a more capable delivery system, productive storage,and controlled release properties through encapsulation and entrapment, polymers, and surfaceionic and weak bond attachments[53–55]. *Ganoderma lucidum* (GL) has been known as a medical mushroom and applied to traditional medicine for past centuries. The reaction parameters affecting the particle size and productive reaction such as pH, reaction time, concentration, and temperature were investigated. The results revealed that pH 9, silver concentration of 1 mM, reaction temperature at 85°C, and reaction time of 6 h were the optimal conditions for the synthesis of AgNPs[56].

Green Synthesis of Metal-Based Nanoparticles Mediated by Ganoderma

Many reports suggested that microorganisms including fungus, bacteria, yeast could be utilized for the synthesis of metal-based nanomaterials. The nanoparticles like gold, calcium, silicon, iron, silver, lead etc received enormous attention to their metal bioaccumulation properties to produce metal NPs[57–59]. The fungal material includes polysaccharides, mycelia and proteins are used in the formation of metal nanoparticles. Fungi have intracellular metal uptake capabilities and maximum wall binding abilities because they have high metal tolerance plus bioaccumulation abilities[8]. Mycelia of *G. lucidum* provides effective hold ability in the bioreactor as well as in agitation and high flow pressure. Hyphae of this species secrete extracellular enzymes in high amounts, leading to the massive production of enzymes. Reduction of the enzyme, using both intracellular and extracellular ways, help in metal NP synthesis, nanostructure, and biomimetic mineralization. This method includes synthesis of NPs inside the fungal cells by transporting ions during the exposure of enzymes[60]. the mycelia cultures are treated with a metal precursor and then they are incubated in the dark for 24 h. For intracellular identification, mycelia are resuspended in phosphate buffer saline (PBS, pH 7.4) and homogenized with a sonicator. NPs formed by the intracellular technique have a smaller size when compared with the NPs fabricated by the extracellular method[22]. This technique is slower when compared with the extracellular method for synthesizing metal NPs.

Table 1. Different mushroom species and their nanomaterial synthesis.

Species	Types of nanoparticles	Size (nm)	Chemical used	Reaction time (hour)	Reducing & Stabilizing agents	Temperature (°C)	Morphology	Ref
Ganoderma lucidum	Ag	50	AgNO3	2	Aquous extract	80	spherical	[21]
Ganoderma spp.	Au	18	HAuCl4. 3H2O	12	Aquous extract	37	spherical	[2]
Ganoderma spp.	Ag	5-50	AgNO3	48	Aquous extract	25	spherical	[7]

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue III, March 2026

Ganoderma spp.	Au	25	HAuCl4. 3H2O	24	Aquous extract	29	spherical	[6]
Ganoderma spp.	Zn	15	ZnS-N3	2	Aquous extract	4	spherical	[18]
Ganoderma spp.	ZnS	2-5	znCl2	24	Aquous extract	70	crystaline	[17]
Ganoderma spp.	TiO2	20	TiCl4	1	Aquous extract	37	spherical	[21]
Ganoderma spp.	ZnO	70-80	Zn(NO3) 2.5H2O	24	Aquous extract	37	spherical	[12]
Ganoderma spp.	Ag	6-10	AgNO3	24	Aquous extract	60	spherical	[7]

Figure 1. Graphical representation for formation of green synthesis of Ganoderma nanomaterials.

Various types of metal nanoparticles

AgNPs play a significant character in the areas of biological and medical sciences. These NPs could be synthesized by various methods, such as physical, chemical, ionizing radiation methods. all of these methods possess potential drawbacks; particularly, the chemicals utilized in AgNP synthesis through wet chemistry routes are less eco-friendly, expensive, and have high toxicity. The filtrate was freeze-dried to prepare aqueous extract[15]. Various concentrations of this aqueous extract were incubated with AgNO3 solution to synthesize AgNPs by the reduction of Ag+ ions to Ag◦ metal. Synthesis of AgNPs was carried using mushroom extract and 1 mM AgNO3 solution. The mixture of solutions was stirred at 90 ◦C for 2 h. Cubical and spherical shaped AgNPs, with an average size of 50 nm, were obtained as a black powder. The synthesized spherical shaped AgNPs with the help of aqueous extract of mushroom (5 mL) and mixed with 95 mL silver nitrate (1 mM, AgNO3) solution to reduce Ag+ to Ag^0. This solution was kept in an incubator for 3 days at 37 ◦C, resulting in color change from light yellow to yellowish-brown[16]. The obtained AgNPs were crystalline with a size ranging from 5 to 25 nm. AuNPs synthesis was performed by using edible mushroom by the photo-irradiation method. The chopped pieces were added in 500 mL of double-deionized water, under stirring, for half an hour. These contents were then incubated overnight. That content was then filtered via filter paper. Later, the filtrate of mushroom was used to reduce Au+ into Au◦ in the presence of bright sunlight to form spherical to triangular-shaped AuNPs in the range of 10–50 nm. ZnS NPs were fabricated using mushroom extract. ZnCl2 and Na2S solution as the precursor material. Small pieces of mushrooms were boiled and filtered. Then, different concentrations of the resultant filtrate were mixed with aqueous solutions of ZnCl2 and Na2S solution, and resulting solutions were dried at 120 ◦C for 2 h. Here, the resultant filtrate was used as a stabilizing (as well as a capping) agent for the fabrication of spherical shaped ZnS NPs. Obtained ZnS NPs was highly crystalline with sizes varying from 2.30 nm to 4.04 nm[17]. ZnONPs were synthesized by using mushroom extract, 20 mL of mushroom extract added into 80 mL of Zn (NO3)2. The 5H2O (5 mM) solution was continuously mixed for 24 h at room temperature until the color transformed into light pink, which confirmed the synthesis of ZnONPs. The multiple characteristics of Cadmium sulphide nanoparticles quantum dots are high photostability, symmetric, slow decay rates, fine emission spectra, wide absorption cross-sections, and broad absorption spectra. TiO2 NPs were synthesized by using edible P. djamor mushroom and evaluated for anticancer potential against A-549 (human lung carcinoma) cell lines, as well as for larvicidal and bactericidal activity. Initially, 10 g of fresh biomass of mushroom was washed with deionized water for 10 min and then cut to small pieces. Later, the chopped pieces were added in 100 mL of doubledeionized water, boiled at 60 ◦C for 15 min, and then filtered[14–

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue III, March 2026

18]. Then, 20 mL of filtrate was added to 80 mL of TiCl4 (5 mM) solution, stirred for 2 h, and kept to room temperature for 20 min until the color changed to brown. The intensity of the color of the extract was determined at the wavelength of 345 nm. The synthesized TiO2 NPs formed, spherical in shape, with sizes of 31 nm[22].

Other nanoparticles synthesis

FeNPs were intracellularly synthesized by using hypha of Pleurotus sp. The reduction process is involved in uptake of FeNPs via the fungal cell membrane, in which reduction of ferric ion (Fe+3) to ferrous ion (Fe+2) takes place. The reduction process is involved during the iron uptake by fungi. These NPs have anticancer activity, excellent bioavailability, and low toxicity. SeNPs have been recorded for inhibiting the proliferation of human breast carcinoma MCF-7 cells by apoptosis[25]. Results obtained from the study revealed that cytotoxicity was cancer specific. Monodispersed copper nanoparticles (CuNPs) were synthesized from aqueous fermented fenugreek powder (FFP), polysaccharides, such as chitosan, sodium alginate, citrus, and pectin, with the help of fungal strains under the exposure of gamma radiation[26].

Table 2. Application of nanoparticles derived from different mushrooms.

Nanoparticles	Applications	References
Au NPs	Anticancer, antioxidant, Antibacterial, Anticandidal	[61,62]
ZnS NPs	Antioxidant, antimicrobial, food packaging	[63]
CdS NPs	Antibacterial, anticancer, nanosensors	[64–66]
Ag NPs	Anticandidal, antifungal, anticancer, photocatalytic	[61]

Applications of plant mediated nanoparticles

Antimicrobial activity

Metal nanoparticles (MNPs) are known to possess potent antimicrobial activity against a wide variety of microbes, including bacteria and fungi, via their photodynamic effects and strong oxidative stress. Metal NPs can also act as photoabsorber material upon excitation of light (most often NIR), resulting in cell death[21]. The photothermal effect comes in origin when the emitted electrons from a higher energy state returns to a low energy state, and release their energy in the form of heat and vibrational energy metal NPs can also act as photoabsorber material upon excitation of light (most often NIR), resulting in cell death. The photothermal effect comes in origin when the emitted electrons from a higher energy state returns to a low energy state, and release their energy in the form of heat and vibrational energy[67].

Anticancer effect

Metal NPs derived from fungi and other sources have been known to possess outstanding anticancer activity because of their profound ROS generation ability under the dark and light exposure. Fabricated Au NPs, 12–15 nm spherical size derived from mushroom extract via the photo-irradiated method and evaluated their anticancer activity against the A-549, MDA-MB, HeLa, and K-562 cell lines[19,20]. The prepared AuNPs showed concentration-dependent activity against all cell lines in between 10 and 30 µg/mL. PS extract and Au NPs, and the reason behind the mechanism was due to the generation of more ROS, leading to oxidative stress, resulting in undeviated damage of protein functionality and integrity. The anticancer activity of TiO2 NPs showed potential toxic effect against human lung cancer (A549) cell lines with maximum inhibited growth of 64% at concentration of 100 µg/mL, after 24 h of exposure[18].

Larvicidal activity

The treating of TiO2 NPs on IVth instar larvae of Ae. aegypti and Cx. quinquefasciatus resulted in larvicidal activity with LC50 (5.88 and 4.84 µg/L) and LC90. The Ae. aegypti larvae treated with ZnONPs showed morphological alteration in the digestive tract, wrecked membrane, midgut, and severe damaging of the brush border, cortex with hyperplasia of gut epithelial cells, and variations in the cytoplasmic masses[17]. The larvae

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of Cx. quinquefasciatus showed the complete putrefaction of abdominal parts, specifically in the caeca, mid-gut, and epithelial layer.

Antidiabetic activity

AgNPs synthesized from P. giganteus possess good α-amylase inhibition activity, which helps in making diabetic drugs inhibition percentage can be increased with increasing concentration of biosynthesized AgNPs. The antidiabetic activity was investigated in vitro through the inhibition of α- amylase, an enzyme that digests starch[21,61,63].

Catalytic activity

Recent research depicted that the rate of the reaction rose with the rise in the loading of the catalyst, and decreased in particles size, clearly reflecting the catalytic behavior of gold nanoparticles against aromatic compounds, resulting in amino-compounds[58,68]. AgNPs (8–35 nm, spherical) loaded on perlite (sheet-like) using Hamamelis virginiana leaf extract and evaluated their catalytic activity against the 4-nitrophenol and Congo red (CR) dye. The authors demonstrated that, with the rise in the concentration of NaBH4 and AgNPs/perlite, the degradation time of 4-nitrophenol decreases, respectively[6]. The AgNPs supported on the surface of perlite facilitate the electron relay from BH4- to 4-nitrophenol as well as CR dye. Furthermore, they claimed that AgNPs/perlite showed high stability and could be used up to 4 times with significant degradation efficacy[16].

CONCLUSION

Global consumption of *G. lucidum* is high, and a large, increasing series of patented and commercially available products that incorporate *G. lucidum* as an active ingredient are available as food supplements[69]. These include extracts and isolated constituents in various formulations, which are marketed all over the world in the form of capsules, creams, hair tonics, and syrups. Human experimental studies have often been small [70] and the results are not always supportive of the in vitro findings. Now, the great wealth of chemical data and anecdotal evidence on the effects of *G. lucidum* needs to be complemented by reliable experimental and clinical data from well-designed human trials in order to clearly establish if the reported health-related effects are valid and significant.

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