Evaluation of antimicrobial, Cytotoxic and genotoxic activities of *Ganoderma lucidum* (Reishi mushroom)

Bülent Ergun
Department of Toxicology, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

Abstract: *Ganoderma lucidum* (GL) is a mushroom used as a traditional remedy for the treatment of various infections since ancient times. This study, was aimed to investigate antimicrobial activity potential of GL against *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Candida glabrata, Candida krusei*, and *Candida parapsilopsis*. Furthermore, it was also aimed to evaluate the toxicity potential of GL. Antimicrobial activities were screened by using microbroth dilution method. With regard to toxicity studies, cytotoxicity was evaluated by using XTT method against NIH3T3 cell lines, whereas genotoxicity study was conducted by Ames MPF 98/100 mutagenicity assay. Obtained data indicated that minimal inhibitory concentration values of the extract against the tested microorganisms ranged from 200 to 400µg/ml. No cytotoxic activity was observed related to the *Ganoderma lucidum* administrations. However, results of the Ames test pointed out a genetic damage with metabolic activation against TA98. At the highest concentration (5mg/ml) the extract showed 2.71 fold increase over the baseline significantly. ($p<0.05$). In conclusion, in spite of significant antimicrobial effect potential, *Ganoderma lucidum* should be used carefully because of its genotoxicity potential.

Keywords: *Ganoderma lucidum*, antimicrobial, cytotoxicity, genotoxicity.

INTRODUCTION

Ganoderma lucidum (also known as Ling-Zhi or Reishi), is a medicinal mushroom, traditionally used for the treatment of several disorders, especially in Asian countries. This mushroom has been used for the treatment of various cardiovascular (hypertension, hyperlipidemia, atherosclerosis), gastrointestinal (hepatic failure, chronic hepatitis, gastric ulcer), psychological/neurological (insomnia, anorexia, neurasthenia), inflammatory (arthritis, bronchitis, nephritis, asthma) and metabolic (diabetes) disorders, as well as infection diseases. Studies on this basidiomycete mushroom have pointed out numerous promising pharmacological effects and then, *Ganoderma lucidum* become a widely used dietary supplement (Boh *et al*., 2007; Batra *et al*., 2013; Hsu *et al*., 2013; Suarez-Arroyo *et al*., 2013; Watanabe *et al*., 2013; You *et al*., 2013). Dietary fibers, mineral elements (zinc, copper, iodine, selenium, and iron), polysaccharides, oligosaccharides, peptides and proteins, amino acids alcohols and phenols, triterpenoids and vitamins are major phytochemical constituents of *Ganoderma lucidum* (Sanodiya *et al*., 2009; Batra *et al*., 2013). Among them, triterpenoids of *Ganoderma lucidum* seem as important active constituents, because of their anti-histaminic, anti-hypertensive, hepatoprotective, hypocholesterolemic, anti-angiogenic/anti-tumor and platelet aggregation inhibitory activities (Boh *et al*., 2007). As well as triterpenoids, polysaccharides may also accepted as another important active constituents of *Ganoderma lucidum* since them, especially β-D-glucans possess anti-tumor activity via immunomodulation and anti-angiogenesis (Boh *et al*., 2007; Suarez-Arroyo *et al*., 2013).

Nowadays, phytotheraphy managements, in other words, treating various diseases by using herbs or herbal preparations, seem as a hot topic in the popular cultures. Recent reports of the World Health Organization pointed out that, 70-80% of the world's population appeals to plant-derived traditional treatment methods for the solution of health problems (*Ahmad et al*, 2006; Shirwaiker *et al*., 2009). Many people accepted plants as "natural" and "safe for health" and unfortunately they are totally unaware of the potential risks. Therefore, investigating the pharmacological (efficacy, mode of action etc.) and toxicological features (safe dose, toxicity profile etc.) of long-term used traditional remedies is of a clinical interest (Flückiger-Isler and Kamber, 2012).

Based on this knowledge, in this study, it was aimed to prepare an extract from *Ganoderma lucidum* and to investigate antimicrobial activity potential of this extract on various microorganisms. Moreover, due to the world-wide extensive use of this *Ganoderma lucidum*, it was also planned to evaluate toxicity potential of the extract prepared from this mushroom.

MATERIALS AND METHODS

Materials

Staphylococcus aureus (ATCC 25923), *Enterococcus faecalis* (ATCC 29212, USA), *Enterococcus faecalis* (ATCC 51922, USA), *Listeria monocytogenes* (ATCC

**Corresponding author:* e-mail: bergun@anadolu.edu.tr

7644), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853, USA), *Escherichia coli* (ATCC 35218), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 90028, USA), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258, USA), and *Candida parapsilopsis* (ATCC 22019, USA) bacterial and fungus strains were evaluated.

Extraction method

5g of dry mushroom material (*Ganoderma lucidum*) was added into 500mL boiled water for 5min to get 1% extract from the material. After 5 minutes the extract was filtered and freeze-dried (Kogiannou DAA *et al*., 2013)

Antimicrobial assay

In vitro growth inhibitory effects of final products on *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212, USA), *Enterococcus faecalis* (ATCC 51922, USA), *Listeria monocytogenes* (ATCC 7644), *Klebsiella pneumoniae* (ATCC 13883, USA), *Pseudomonas aeruginosa* (ATCC 27853, USA), *Escherichia coli* (ATCC 35218, USA), *Escherichia coli* (ATCC 25922, USA), *Candida albicans* (ATCC 90028, USA), *Candida glabrata* (ATCC 90030, USA), *Candida krusei* (ATCC 6258, USA), and *Candida parapsilopsis* (ATCC 22019, USA) bacterial and fungus strains were evaluated.

CLSI reference M7-A7 broth microdilution method, described in a previous study (Ozdemir *et al*., 2013), was performed and minimum inhibitory concentrations (MIC) were compared.

Cytotoxicity test

In order to evaluate the possible cytotoxic effect of *Ganoderma lucidum* extract, XTT assay (Xenometrix AG, Switzerland) was carried out in NIH3T3 mouse embryonic fibroblast cell line (ATCC® number CRL-1658™) according to the manufacturer's instructions. Optic density was measured at 480 nm with a reference wave length at 680 nm. Using the obtained results inhibition percentages were calculated for each concentrations of the extract. IC50 values were calculated by plotting a dose response curve of the inhibition% versus test compound concentrations. (Altıntop *et al*., 2012).

Genotoxicity test (Ames MPF ™)

Ames MPF 98/100 mutagenicity assay sample kit (Xenometrix AG, Gewerbertrasse, Switzerland) was used for determining the mutagenicity of the compounds. The experiment was performed according to the kit procedure. Fold induction over the baseline was determined by calculating the ratio of the mean number of positive wells for the dose concentration divided by zero-dose baseline. The zero-dose baseline was obtained after adding one standard deviation to the mean number of positive wells of the zero-dose control. If the baseline was less than 1, the value is set to 1 for calculation. All doses were compared according to Student's t-test at $p<0.05$ for statistical significance. The mutagenicity of the compounds was determined according to the criteria [Flückiger-Isler S and Kamber M, 2012].

STATISTICAL ANALYSIS

Student's t test was performed for statistical analysis and $p<0.05$ was accepted as significant.

RESULTS

Antimicrobial assay

Antimicrobial effect of *Ganoderma lucidum* extract was tested *in vitro* on various bacteria and fungus species. Among them, *Enterococcus faecalis* (ATCC 51922, USA) and *Pseudomonas aeruginosa* (ATCC 27853, USA) were the most susceptible microorganism to *Ganoderma lucidum* extract. MIC value was found as 200µg/mL on *Enterococcus faecalis and* 400µg/mL for *Pseudomonas aeruginosa* strains for both extract and chloramphenicol. According to our results, effective antibacterial dose of the extract on *Enterococcus faecalis* and Pseudomonas aeruginosa is much lower than its cytotoxic effective dose on NIH3T3 cells (IC_{50} >500).

Antimicrobial activity test results of *Ganoderma lucidum* extract and reference drugs are presented in table 1.

Cytotoxicity test

IC_{50} value was found as >500µg/ml for *Ganoderma lucidum* extract on NIH3T3 cells. In other words, *Ganoderma lucidum* extract did not have any cytotoxic activity on a healthy embryonic fibroblast cell line, NIH3T3 cells.

Genotoxicity test (Ames MPF ™)

Results of the AMES tests were presented in table 2. Our experiments exhibited that, *Ganoderma lucidum* extract showed a baseline of 6.12 against TA 100 without S9. At the concentration of 0.31mg/ml, it showed a 1.69 fold increase over the baseline significantly. But there were not any adjacent doses showing any significance or any significant increase at the highest non-toxic dose level. According to the criteria above, *Ganoderma lucidum* extract can be classified as negative against TA 100 without S9. Besides, obtained data against TA 100 with S9 indicated that, *Ganoderma lucidum* extract showed a baseline of 7.42. There were no significant increases above 1.5-2.5 fold increase over the baseline. However, it did not show a dose-response tendency. Therefore, *Ganoderma lucidum* extract can be classified as negative against TA 100 with S9.

Table 1: MIC values (µg/mL) of chloramphenicol, ketoconazole and *Ganoderma lucidum* extract on different microorganisms.

Microorganism	GLE	Chloramphenicol	Ketoconazole	Microorganism	GLE	Chloramphenicol	Ketoconazole
A	200	25	ND	G	400	100	ND
B	200	12,5	ND	H	400	12,5	ND
C	200	200	ND	I	400	ND	100
D	400	6,25	ND	J	400	ND	200
E	400	50	ND	K	400	ND	25
F	400	400	ND	L	400	ND	200

Definition of abbreviations: GLE: *Ganoderma lucidum* extract, MIC: Minimum inhibitory concentration, ND: Not determined, A: *Staphylococcus aureus* (ATCC 25923), B: *Enterococcus faecalis* (ATCC 29212), C: *Enterococcus faecalis* (ATCC 51922), D: *Listeria monocytogenes* (ATCC 7644), E: *Klebsiella pneumoniae* (ATCC 13883), F: *Pseudomonas aeruginosa* (ATCC 27853), G: *Escherichia coli* (ATCC 35218), H: *Escherichia coli* (ATCC 25922), I: *Candida albicans* (ATCC 90028), J: *Candida glabrata* (ATCC 90030), K: *Candida krusei* (ATCC 6258), L: *Candida parapsilopsis* (ATCC 22019).

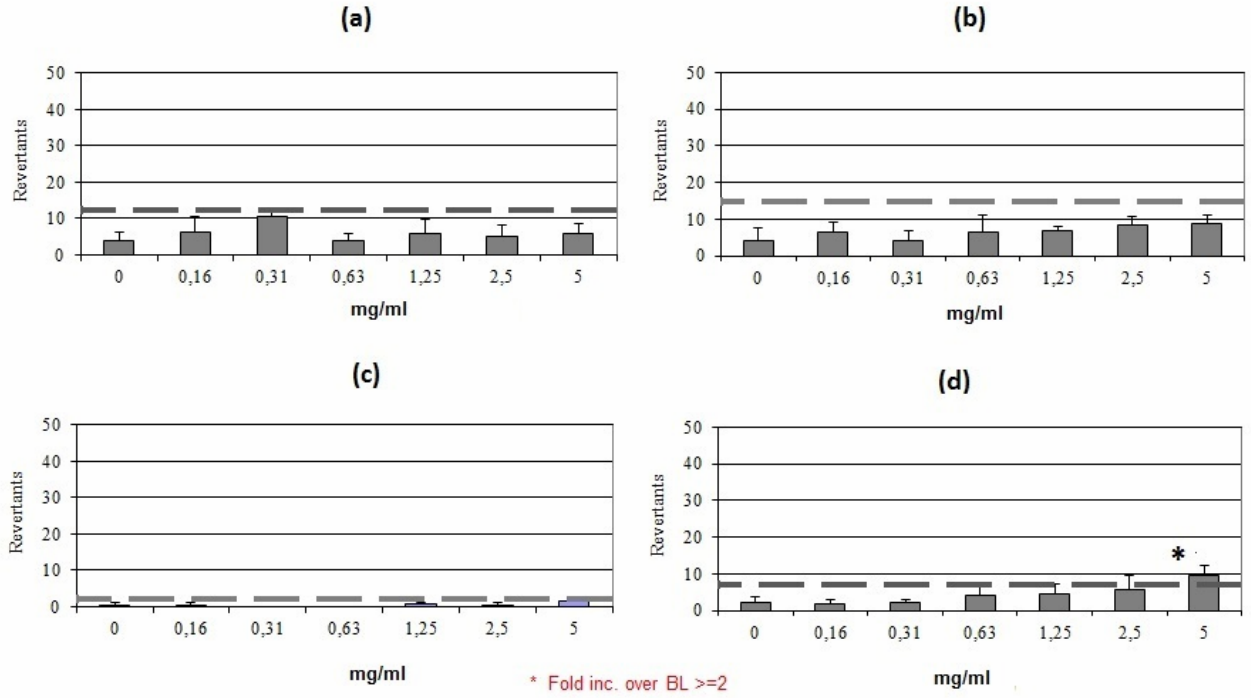

Fig. 1: Dose response curve of *Ganoderma lucidum* extract with TA98 and TA100 in presence of S9 mix and in absence of S9 mix. (a) strain TA 100 without S9 mix; (b) strain TA 100 with S9 mix; (c) strain TA98 without S9 mix; (d) strain TA98 with S9 mix, *p<0.05.

Ganoderma lucidum extract showed a baseline of 1.04 against TA 98 without S9. There were no significant increases above 2-3 fold increase over the baseline. Also, it did not show a dose-response tendency. According to the criteria above it can be classified as negative against TA 98 without S9. On the other hand, obtained data against TA 98 with S9 indicated that *Ganoderma lucidum* extract showed a baseline of 3.56. At the highest concentration (5mg/ml) the extract showed 2.71 fold increase over the baseline significantly. Besides, the adjacent dose (2.5mg/ml) showed a significant increase and *Ganoderma lucidum* extract shows dose-response tendency. According to the criteria above, *Ganoderma lucidum* extract can be classified as possibly positive against TA 98 in the presence of S9 (fig. 1).

DISCUSSION

In this study, based on the folkloric usage and therapeutic potential of *Ganoderma lucidum* on infection diseases, it was planned to investigate antimicrobial activities of this mushroom by using various microorganisms. Moreover, based on the world-wide extensive use of this mushroom, it was also aimed to evaluate toxicity potential of the prepared extract.

Antimicrobial activities of *Ganoderma lucidum* extract were screened by using microbroth dilution method. In our study, similar to reference drug chloramphenicol, tested extract showed antimicrobial effect against *Enterococcus faecalis* and *Pseudomonas aeruginosa*.

Obtained data indicated that MIC values of the extract prepared from this mushroom against the tested microorganisms ranged from 200 to 400µg/ml. These results are matched with previous works reporting the antimicrobial activity of various extracts prepared from *Ganoderma lucidum* (Karwa and Rai, 2012) and suggesting the synergistic bactericidal effect of commercial antibiotic therapy combining with *Ganoderma lucidum* extract (Ulbricht *et al*., 2010). Although, active component/components of *Ganoderma lucidum* extract has not been identified yet, triterpenoids and/or polysaccharides may be responsible from the observed antimicrobial effect.

In spite of world-wide usage *Ganoderma lucidum*, especially for treatments of leukemia, carcinoma, hepatitis and diabetes as an alternative adjuvant drug (Shirwaiker *et al*., 2009), little is known about the effectiveness and safety of this mushroom. Some recent studies have not confirmed the findings of the previous papers reporting the beneficial pharmacological effects of *Ganoderma lucidum*. Rather, some studies and case reports have pointed out a notable hepatotoxic potential induced by this mushroom (Heleno *et al*., 2013). Based on this information, together with the world-wide extensive use of *Ganoderma lucidum*, cytotoxicity and genotoxicity potential of the extract prepared from this mushroom was also examined in this study.

Cytotoxicity may be defined as "being toxic to cells". For evaluating cytotoxicity, harmful or unwanted effect may induced by using different chemical agents in cell culture systems. Cytotoxicity testing includes numerous methods, both qualitative and quantitative ones (Bombick *et al*., 1998; Langdon *et al*., 2010). In this study, cytotoxic activity of the extract was evaluated using XTT method, which is a colorimetric assay for assessing cell metabolic activity. Obtained data indicated that *Ganoderma lucidum* extract has no inhibitory effect on NIH3T3 cell line with IC50 value >500µg/ml. In other words, mitochondrial activity was not affected by *Ganoderma lucidum* extract in NIH3T3 cell line, at the administrated doses. As a notable result, detected antibacterial doses of this extract against *Enterococcus faecalis* and *Pseudomonas aeruginosa* were lower than its cytotoxic dose. On the other hand, it should be noted that, although *Ganoderma lucidum* extract has not a cytotoxic potential against NIH3T3 cell line, it is known to possess a strong cytotoxic effect against cancer cell lines by acting on mitochondrial pathways (Kao *et al*., 2013).

Ames test, mammalian cell mutation test, and *in vitro* micronucleus assays are three of the recommended *in vitro* tests used for evaluating genotoxic potential of the chemical agents (Lorge *et al*., 2007). Among them Ames test, most commonly used mutagenicity test, uses several strains of the bacterium *Salmonella typhimurium* that carry mutations in genes involved in histidine synthesis (Tabrez *et al*., 2011). This test, providing rapid prediction for determining the genotoxicity, is also a comparable method showing high correlation with the rodent mutagenicity (Reifferscheid and Heil, 1996; Kamber *et al*., 2009). In this study, genotoxicity potential of the tested extract was evaluated by using Ames MPF 98/100 mutagenicity assay. Obtained data indicated that *Ganoderma lucidum* extract may have genotoxic effects with metabolic activation against TA 98 at high concentrations (>2,5mg/ml). This finding may be supported by a study of Wachtel-Galor and his co-workers, reporting the occurrence of hydrogen peroxide mediated DNA damage with high concentrations of this mushroom (Wachtel-Galor *et al*., 2005). Further, in another study, *Ganoderma lucidum* preparations have been shown to possess a significant mutagenic potential with and without S9 against TA 100 and with S9 against TA 98 (Madan, 2012). On the other hand, some other previous studies using similar or different assays have not been reported a significant genotoxicity induced by *Ganoderma lucidum* (Chiu *et al*., 2000; Lakshmi *et al*., 2006). These conflicting results may be related to different phytochemical content of the tested *Ganoderma lucidum* extracts or may be caused by the variance of experimental conditions.

In summary, *Ganoderma lucidum* extract have some beneficial health effects, including antimicrobial activity potential, as indicated in this study. Additionally, although this study also provide some data suggesting a genotoxic potential for the *Ganoderma lucidum* extract, we do not have sufficient information with regard to adverse effects and toxicity potential of this mushroom. Therefore, further detailed studies are needed for clarifying this issue.

CONCLUSION

In conclusion, obtained data indicated that MIC values of the extract against various microorganisms tested in this study were ranged from 200 to 400µg/ml and the most susceptible strains to *Ganoderma lucidum* extract were detected as Enterococcus faecalis (ATCC 51922) and *Pseudomonas aeruginosa* (ATCC 27853. Further, *Ganoderma lucidum* extract did not induce any cytotoxic activity on NIH3T3 cells. On the other hand, genotoxicity studies indicated a genetic damage at high concentrations of the extract. According to these results, not only patients but also health professionals should be careful about the uncontrolled consumption of this mushroom. Especially, long-term and high dose exposure should be avoided until the identification of the toxicity profile.

REFERENCES

Ahmad I, Aqil F, Ahmad F and Owais M (2006). Herbal Medicines: Prospects and Constraints, Modern

Phytomedicine: Turning Medicinal Plants into Drug, I. Ahmad, F. Aqil, M. Owais, Wiley-Vch, pp.59-60.

Altıntop MD, Özdemir A, Turan-Zitouni G, Ilgın S, Atlı Ö, İşcan G and Kaplancıklı ZA (2012). Synthesis and biological evaluation of some hydrazone derivatives as new anticandidal and anticancer agents. *Eur. J. Med. Chem.,* **58**: 299-307.

Batra P, Sharma AK and Khajuria R (2013). Probing Lingzhi or Reishi medicinal mushroom *Ganoderma lucidum* (higher Basidiomycetes): A bitter mushroom with amazing health benefits. *Int. J. Med. Mushrooms,* **15**: 127-143.

Boh B, Berovic M, Zhang J and Zhi-Bin L (2007). *Ganoderma lucidum* and its pharmaceutically active compounds. *Biotechnol. Annu. Rev.*, **13**: 265-301.

Bombick DV, Putnam K and Doolittle DJ (1998). Comparative cytotoxicity studies of smoke condensates from different types of cigarettes and tobaccos. *Toxicology in vitro*, **12**: 241-249.

Flückiger-Isler S and Kamber M (2012). Direct comparison of the Ames microplate format (MPF) test in liquid medium with the standard Ames preincubation assay on agar plates by use of equivocal to weakly positive test compounds. *Mutation Res.*, **747**: 36-45.

Heleno SA, Ferreira IC, Esteves AP, Ćirić A, Glamočlija J, Martins A, Soković M and Queiroz MJ (2013). Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food. Chem. Toxicol.,* **58**: 95-100.

Hsu HY, Kuan YC, Lin TY, Tsao SM, Hsu J, Ma LJ and Sheu F (2013). Reishi protein LZ-8 induces FOXP3(+) treg expansion via a CD45-dependent signaling pathway and alleviates acute intestinal inflammation in mice. *Evid. Based Complement. Alternat. Med.,* **2013**: 513-542.

Kamber M, Flückiger-Isler S, Engelhardt G, Jaeckh R and Zeiger E (2009). Comparison of the Ames II and traditional Ames test responses with respect to mutagenicity, strain specificities, need for metabolism and correlation with rodent carcinogenicity. *Mutagenesis,* **24**: 359-366.

Kao CHJ, Jesuthasan AC, Bishop KS, Glucina MP and Ferguson LR (2013). Anti-cancer activities of *Ganoderma lucidum*: Active ingredients and pathways. *FFHD*, **3**: 48-65.

Karwa AS and Rai MK (2012). Naturally occurring medicinal mushroom-derived antimicrobials: a case-study using Lingzhi or Reishi *Ganoderma lucidum* (W. Curt.:Fr.) P. Karst. (higher Basidiomycetes). *Int. J. Med. Mushrooms,* **14**: 481-490.

Kogiannou DAA, Kalogeropoulos N, Kefalas P, Polissiou MG and Kaliora AC (2013). *Herbal infusions; their phenolic profile, antioxidant and anti-inflammatory effects in HT29 and PC3 cells*. Food and Chemical Toxicology, **61**: 152-159.

Langdon SR, Mulgrew J, Paolini GV and VanHoorn WP (2010). Predicting cytotoxicity from heterogeneous data sources with bayesian learning, *J. Cheminform.,* **2**: 1-18.

Lorge E, Gervais V, Becourt-Lhote N, Maisonneuve C, Delongeas JL and Claude N (2007). Genetic toxicity assessment: Employing the best science for human safety evaluation part IV: a strategy in genotoxicity testing in drug development: some examples. *Toxicol. Sci.,* **98**: 39-42.

Ozdemir A, Altintop MD, Kaplancikli ZA, Turan-Zitouni G, Karaca H and Tunali Y (2013). Synthesis and biological evaluation of pyrazoline derivatives bearing an indole moiety as new antimicrobial agents. *Arch Pharm (Weinheim),* **346**: 463-469.

Reifferscheid G and Heil J (1996). Validation of the SOS/umu test using test results of 486 chemicals and comparison with the Ames test and carcinogenicity data. *Mutation Res.,* **369**: 129-145.

Sanodiya BS, Thakur GS, Baghel RK, Prasad GB and Bisen PS (2009). *Ganoderma lucidum*: A potent pharmacological macrofungus. *Curr. Pharm. Biotechnol.,* **10**: 717-742.

Shirwaiker A, Verma R, Lobo R and Shirwaiker A (2009). Phytotherapy-safety aspects. *Nat. Prod. Rad,.* **8**: 55-63.

Suarez-Arroyo IJ, Rosario-Acevedo R, Aguilar-Perez A, Clemente PL, Cubano LA, Serrano J, Schneider RJ and Martínez-Montemayor MM (2013). Anti-tumor effects of *Ganoderma lucidum* (reishi) in inflammatory breast cancer *in vivo* and *in vitro* models. *PLoS One*, **8**: e57431.

Tabrez S, Shakil S, Urooj M, Damanhouri GA, Abuzenadah AM and Ahmad M (2011). Genotoxicity testing and biomarker studies on surface waters: an overview of the techniques and their efficacies. *J. Environ. Sci. Health C. Environ. Carcinog. Ecotoxicol. Rev.,* **2**: 250-275.

Ulbricht C, Abrams TR, Bent S, Boon H, Costa D, Dacey C, Guilford J, Giese N, Grimes Serrano JM, Hackman DA, Scully L, Rusie E, Shaffer M, Varghese M, Vijarian N, Weissner W, Welch S, Wong D and Woods J (2010). Reishi Mushroom (*Ganoderma lucidum*): Systematic review by the natural standard research collaboration. *J. Soc. Integr. Oncol.,* **8**: 148-159.

Watanabe H, Kashimoto N, Ushijima M and Tamura K (2013). Effects of a water-soluble extract of *Ganoderma lucidum* mycelia on aberrant crypt foci induced by azoxymethane and small-intestinal injury by 5-FU in F344 rats. *Med. Mol. Morphol.,* **46**: 97-103.

You BJ, Lee MH, Tien N, Lee MS, Hsieh HC, Tseng LH, Chung YL and Lee HZ (2013). A novel approach to enhancing ganoderic acid production by *Ganoderma lucidum* using apoptosis induction. *PLoS One,* **8**: e53616.