Received: 12 December 2018 | Revised: 19 March 2019 | Accepted: 15 April 2019

DOI: 10.1002/ptr.6384

REVIEW

WILEY

The pharmacological potential and possible molecular mechanisms of action of *Inonotus obliquus* from preclinical studies

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Funding information
Russian Science Foundation, Grant/Award Number: 16-15-000390-П; Ministry of Education of the Russian Federation, Grant/Award Number: 4.9514.2017/8.9; Program 211 of the Government of the Russian Federation, Grant/Award Number: 02.A03.21.0006; Russian Foundation for Basic Research, Grant/Award Numbers: 17-03-00641 and 18-29-12129mk

The use of mushrooms as functional foods and in the treatment of diseases has a long history. *Inonotus obliquus* is a mushroom belonging to the *Hymenochaetaceae* family and has possible anticancer, antiviral, and hypoglycemic properties. Chemical analysis of this mushroom has allowed the identification of various constituents such as melanins, phenolic compounds, and lanostane-type triterpenoids. A plethora of findings have highlighted the potential molecular mechanisms of actions of this mushroom such as its ability to scavenge reactive oxygen species, inhibit the growth of tumors, decrease inflammation and insulin resistance in type 2 diabetes, and stimulate the immune system. This review summarizes the relevant findings with reference to the therapeutic potential of this mushroom in countering the progression of cancers, diabetes mellitus, and antiviral activities, while highlighting its possible molecular mechanisms of action. The possible role of this mushroom as a therapeutic agent in addressing the pathogenesis of diabetes and cancer has also been suggested.

KEYWORDS

anticancer, antiviral, immunostimulatory, inflammation, *Inonotus obliquus*, type 2 diabetes

1 | INTRODUCTION

The use of mushrooms as a functional food and in the treatment of various diseases dates back to the evolution of man (Blagodatski et al., 2018). These spore-bearing fruiting bodies of fungi serve as a rich source of vitamins, carbohydrates, protein, minerals, and secondary metabolites. In addition, they also contain different bioactive molecules such as polysaccharides, phenols, terpenoids, steroids, glycoprotein derivatives, and nucleotides that are capable of regulating metabolic processes and supporting overall better health (Yang, Lin, & Mau, 2002). *Inonotus obliquus* (*I. obliquus)* is a mushroom belonging to the family Hymenochaetaceae (Basidiomycota) popularly called chaga in Russian folk medicine. This mushroom is widely consumed as a food and made into tea, syrup, bath agents, or concentrate for its beneficial health properties (Kim et al., 2006; Rhee, Cho, Kim, & Cha, D.S. And Park, H.J., 2008). It is typically grown at the latitudes of 45–50°N and has been employed in the treatment of cardiovascular disease, gastrointestinal cancer, and diabetes mellitus (DM) in Russia and other East-European countries (Cui, Kim, & Park, 2005; Sun, Ao, & Lu, 2008).

Chemical analysis of *I. obliquus* reveals that this mushroom contains over 200 different types of bioactive substances (Liu et al.,

Abbreviations: AGE, advanced glycation end-products; Akt, protein kinase B; CAT, catalase; CDK, cyclin-dependent kinase; COX-2, cyclooxygenase-2; CRP, C-reactive protein; DDC, diethyldithiocarbamate; DM, diabetes mellitus; DPP-4, dipeptidyl peptidase 4; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GSH, reduced glutathione; HDL, high-density lipoprotein; HFD, high-fat diet; *I. obliquus*, *Inonotus obliquus*; IBD, inflammatory bowel disease; IC_{50}, The half maximal inhibitory concentration; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; *IOGBR*, *I. obliquus* grown on germinated brown rice; IOP, *I. obliquus* polysaccharides; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinases; MDA, malondialdehyde; MMP, matrix metalloproteinase; PGE2, Prostaglandin E2; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; SGLT2, sodium-glucose cotransporter 2; SOD, superoxide dismutase; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TGF, transforming growth factor; TNF-α, tumor necrosis factor alpha; TZD, thiazolidinedione; WAT, white adipose tissues

2014). Of these, *I. obliquus* polysaccharides (IOP) are considered as the most bioactive ingredients due to their hypoglycemic, antioxidant, and antiinflammatory activities (Choi et al., 2010; Ma, Chen, Zhang, Zhang, & Fu, 2012). Extracts of *I. obliquus* have been shown to counter the action of free radicals and hamper oxidative stress in lymphocytes of inflammatory bowel disease patients (Najafzadeh, Reynolds, Baumgartner, Jerwood, & Anderson, 2007), retard the proliferation of human colon cancer cells (Lee, Hwang, & Yun, 2009), and impede the expression of inflammatory mediators in murine macrophage cell lines (Choi et al., 2010; Kim et al., 2007). Several authors have reported that polysaccharide extracts of *I. obliquus* effectively restored abnormal levels of oxidative indices in streptozotocin (STZ)-induced diabetic rats (Diao, Jin, & Yu, 2014) and diminished pancreatic acinar atrophy in diethyldithiocarbamate-induced mice (Hu et al., 2016). Despite the plethora of potential health benefits associated with *I. obliquus*, its possible molecular mechanisms of action have not been extensively studied.

This review is aimed at understanding relevant findings with respect to the potential therapeutic properties of *I. obliquus* as antidiabetic, anticancer, antiviral, and immunostimulating agents, while concomitantly highlighting the possible molecular mechanisms involved.

2 | METHOD

A scoping review method was employed to dissect and analyze relevant literature. The methodological scheme first proffered by Arksey and O'Malley (2005) and later revised by Levac, Colquhoun, and O'Brien (2010) was utilized for this study. The key research question of this study was the following: What are the therapeutic effects of *I. obliquus* extracts on disease conditions or disorders such as DM, cancer, viral infections, inflammation, and oxidative stress in animal or cell line studies?

2.1 | Search strategy

A methodical literature search from 1990 to 2018 was performed using the following terms "*I. obliquus* OR "*I. obliquus* polysaccharides" OR "Chaga mushroom" "combined with diabetes mellitus" OR "type 2 diabetes mellitus" OR "T2DM" OR "insulin resistance" OR "β-cell proliferation" OR "oxidative stress" OR "inflammation" OR "adiposity" OR "cancer" OR "antiviral" OR "immune stimulation" on the PubMed, Scopus, Science Direct, and Web of Science databases.

2.2 | Selection criteria

The literature search yielded a total of 680 abstracts of articles. The formulation process of the selection criteria of relevant studies was monotonic and based on the title screening, abstract, and the full text of the articles when required. The 680 articles identified were thoroughly perused by two independent reviewers.

In order for an article to be selected and included in this review, the article must have the following:

1. Been an original study, with data published in a peer-reviewed scientific journal.
2. Addressed the effect of *I. obliquus* treatment on cancer, DM, or viral replication in either animal models or cell cultures.
3. Taken into account the therapeutic molecular mechanisms of action of *I. obliquus* in the treatment of the aforementioned disease conditions.

The reference section of each eligible article was thoroughly screened by authors. Letters to editors, conference proceedings, and chapters of books were excluded from this review.

2.3 | Data extraction and database generation

Out of the 680 articles identified through the search strategy, only 60 articles met the eligibility criteria to be included in this review. Data obtained from the articles following the literature search supported the antidiabetic, anticancer, antiviral, and immunostimulating effects of *I. obliquus*. The individual biologically active components of *I. obliquus* are shown in Table 1, and the antidiabetic effect and the possible anticancer molecular mechanisms of this mushroom in animal and cell studies are shown in Tables 2 and 3, respectively. All articles retrieved from the literature search and used for this review involved either animal or cellular studies. However, no article retrieved from the search strategy highlighted the antidiabetic, antiviral, anticancer, or immunostimulating effect of this mushroom in human studies.

3 | CHEMICAL ANALYSIS OF *I. OBLIQUUS*

The chemical composition of *I. obliquus* was first studied by Dragendorff in 1864; the polysaccharides of this species of mushroom are regarded as the most active compounds, due to an array of biological activities associated with these sugars (Choi et al., 2010; Ma et al., 2012; Shivrina, Lovyagina, & Platonova, 1959). Ludwiczak and Wrecino first detected and identified lanostane triterpene compounds (lanosterol-3β-hydroxy-lanosta-8,24-diene and its derivative inotodiol) in this mushroom (Ludwiczak & Wrecino, 1962). Kahlos and his coworkers isolated β-hydroxylanosta-8,24-dien-21-oic acid (trametenolic acid), 3β-hydroxylanosta-8,24-dien-21-al, 3β,22,25-trihydroxylanosta-8,23-diene, and d 3β,22-dihydroxylanosta-8,24-dien-7-one from this mushroom (Kahlos & Hiltunen, 1983; Kahlos, Hiltunen, & Schantz, 1984). Recently, chagabusone, a lanostane-type triterpenoid, was isolated following the fractionation of methanolic extracts of this mushroom (Baek et al., 2018). Careful examination of the sclerotia of this mushroom by Shin et al. led to the isolation of 3β-hydroxylanosta-8,24-diene-21,23-lactone, 21,24-cyclopentalanost-8-ene-3β,21,25-triol, and lanost-8-ene-3β,22,25-triol (Shin, Tamai, & Terazawa, 2000;

Shin, Tamai, & Terazawa, 2001). Inonotsutriols D and E were equally isolated from the sclerotia of this mushroom (Taji et al., 2007). Currently, approximately 40 triterpene compounds of the lanostane series have been isolated from *I. obliquus*, as well as trace amounts of pentacyclic triterpenes such as betulin, lupeol, and lupenon (Gao, Xu, Lu, & Xu, 2009). Furthermore, steroids, mainly ergosterol and other typically plant-derived steroidal compounds such as sitosterol, and stigmasterol have been identified, whereas the ergosterol content of *I. obliquus* was found to be lower when compared with the triterpene content (Nikitina, Khabibrakhmanova, & Sysoeva, 2016). The β-*D*-glucose polysaccharide (β-glucan) content of *I. obliquus* is also a predominant bioactive compound that has attracted much interest owing to its beneficial properties on health as a prebiotic, and hypoglycemic agent (Ham et al., 2003). *I. obliquus* also contains lectins; some of which contain calcium, magnesium, and other metallic/nonmetallic ions. Melanin, a polyphenolic pigment that is form as a result of the oxidative polymerization of phenols, was also identified following a physicochemical analysis this mushroom (Babitskaya, Shcherba, & Lkonnikova, 2000). The chemical characterization of the aqueous extracts of *I. obliquus* showed that oxalic acid was the most predominant organic acid present among other organic acids such as gallic, protocatechuic, and p-hydroxybenzoic acids (Glamočlija et al., 2015).

This mushroom also contains flavonoids (e.g., flavonols, flavones, catechols, and anthocyanin), hemicellulose (~12.5%), and cellulose (~2%; Shashkina, Shashkin, & Sergeev, 2006). The chemical evaluation of some of the bioactive constituents of *I. obliquus* is shown in Table 1.

4 | INFLUENCE OF *I. OBLIQUUS* EXTRACTS ON SERUM GLUCOSE, INSULIN SECRETION, AND PANCREATIC B-CELLS

DM is a major global threat to human health because of its growing prevalence and deleterious complications. The recent global estimate of people living with this disease is approximately 425 million, and this figure is expected to surge to 629 million by 2045 (IDF, 2017). Type 2 DM (T2DM) primarily arises from inadequate glycemic control, a condition that usually occurs as a result of relative deficiency of and resistance to the pancreatic hormone, insulin. Postprandial hyperglycemia plays a crucial role in the pathogenesis and complications associated with this disease; hence, effective control of elevated blood glucose levels following meals is key in the management of T2DM. To maintain normoglycemia in insulin-resistant states, pancreatic islets usually respond by enhancing insulin secretion. Although the molecular mechanisms involved are not well understood, it is evident from animal studies that the expansion of both β-cell mass and improved β-cell function is crucial in proper insulin secretion (Liu, Jetton, & Leahy, 2002; Steil et al., 2001). Pancreatic β-cells play a pivotal role in the regulation of carbohydrate, protein, and fat metabolism as well as in the maintenance of energy balance in adipose tissues, skeletal muscles, and the liver. Insulin secretion is extremely sensitive to fluctuation of blood glucose levels, and this is attained by the integration of glucose metabolism with insulin secretion via the variation in intracellular Adenosine triphosphate (ATP) levels, electrical activity of β-cells, and insulin vesicle release. The clinical manifestation of T2DM due to

TABLE 1 Bioactive constituents extracted from *Inonotus obliquus* using different solvents and their potential biological activities

Biological metabolites	Potential biological activities	Extracting solvent	Reference
Inotodiol	Antihyperglycemic; antimutagenic; antioxidative;	Petroleum ether; ethyl acetate	Li et al., 2018; Ham et al., 2009
Ergosterol peroxide	Anticarcinogenic, hypoglycemic	Ethyl acetate	Li et al., 2016; Lu et al., 2010.
Melanin	Hypoglycemic	Water	Lee & Hyun, 2014
3β-Hydroxylanosta-8, 24-diene-21-al	Antimutagenic and antioxidative; anticarcinogenic, hypoglycemic	Ethyl acetate; methanol;	Ham et al., 2009; Chung, Chung, Jeong, & Ham, 2010; Lu et al., 2010.
Hispolon	Suppresses the growth of cells (human epidermoid KB cells)	Ethanol	Chen, He & Li, 2006
Caffeic acid	Antioxidant	Methanol	Nakajima, Sato, & Konishi, 2007
Hispidin	Suppresses tumor growth (HT-29 human colon cancer cells)	Water	Lee et al., 2009.
Lanosta-24-ene-3β, 21-diol	Antitumor activity	Chloroform	Taji et al., 2008
Protocatechuic acid	Antioxidant; antiviral	Methanol	Nakajima et al., 2007
Lanosta-7, 9(11), 23E-triene-3β, 22R, 25-triol	Inhibits tumor proliferation	Chloroform	Taji et al., 2008
Betulinic acid	Antip roliferative effect against human lung adenocarcinoma cells (A549)	Water	Géry et al., 2018

TABLE 2 Anti-diabetic effects of *Inonotus obliquus* in in vivo and in vitro studies

Bioactive component of *I. obliquus* used	Experimental model	Study duration	Dose	Effects	Possible molecular mechanism	Reference
Polysaccharides	HFD/STZ induced T2DM C57/BL/6 mice	8 weeks	300 mg/kg	↑insulin sensitivity, ↓ triglyceride level and improved the HDL/LDL ratio; 3 days of incubation of renal tubular cells (LLC-PK1) with the polysaccharides (100 μg/ml) protected against STZ + AGEs-induced glucotoxicity.	↓ the expression of NF-B/TGF-B pathways.	Chou et al., 2016
Polysaccharide	STZ-induced T2DM Wister rat	6 weeks	30, 20, and 10 mg/kg	↓blood glucose level in dose dependent manner; improved the level of HDL cholesterol; ameliorated the oxidative indices; partially regenerated the STZ-damaged pancreatic-cells	↓the expression of IL-1β, TNF-α.	Diao et al., 2014
	3T3-Li preadipoyte culture		10, 25, 50 and 100 μg/ml	↑ the differentiation of 3T3-Li preadipoyte in dose dependent manner.	↑ the expression of PPARY and GLUT4, but do not exhibit PPARY ligand activity; ↑the expression of C/EBPα;	Joo et al., 2010
Inotodiol	STZ-induced T2DM female Sprague–Dawley rats	18 weeks	10 mg/kg	Exerted antihyperglycemic effect by decreasing the blood glucose, and plasma levels of cholesterol, and triglyceride; Improving the body weight.	↑the level of SOD, GPx and MDA; suppressed the expression of β-catenin pathways.	Zhang, Lin, et al., 2018
Melanin complex	3T3-L1 adipocytes	10 weeks	50 mg/kg	Stimulated insulin sensitive and ↓adiposity in dose dependent manner	↑the phosphorylation of Akt and AMP-activated protein kinase; ↑Adiponectin	Lee & Hyun, 2014

(Continues)

TABLE 2 (Continued)

Bioactive component of *I. obliquus* used	Experimental model	Study duration	Dose	Effects	Possible molecular mechanism	Reference
					gene expression; ↑the *FAS* gene expression but showed no significant influence in expression of several other lipogenic genes (such as the *ACC*, *PPAR-γ*, and *SREBP1-c*)	
Polysaccharides	RINm5F pancreatic cells		50 or 100 μg/ml	↑insulin secretion; exhibited cytoprotective activities by reducing the DNA fragmentation, decreasing the apoptosis rate, and inhibiting the generation of ROS in H_2O_2-treated pancreatic β-cells.	↓ the phosphorylation of MAPKs/ERK pathways but showed no effect on the phosphorylation of JNK pathways.	Sim et al., 2016
	Male ICR diabetic mice	3 weeks	30 and 60 mg/kg	Amended pancreatic tissues damage; ↓ the serum levels of LDL-cholesterol, total cholesterol, and triglycerides; ↑ insulin, HDL-cholesterol, and hepatic glycogen levels.	↑ activities of CAT, SOD and GPx; ↓the activities of MDA in the liver.	Xu et al., 2010

inadequate glycemic control is a consequence of the deficiency of and resistance to insulin.

It has been reported that the treatment of STZ-induced diabetic Wistar rats with polysaccharide extracts of *I. obliquus* at doses of 10, 20, and 30 mg/kg for 6 weeks reduced blood glucose levels in a dose-dependent manner and restored the structure of β-cells after diabetic-induced cellular damage (Diao et al., 2014). Eight weeks of dietary treatment with fermented *I. obliquus* lowered serum glucose and leptin levels and alleviated obesity-related complications in T2DM OLETF rats (Cha, Jun, & Yoo, 2006). The dry matter extracts obtained from culture broth of *I. obliquus* were found to improve serum insulin levels, moderately expand the pancreatic islets, and reduce pancreatic injuries in alloxan-induced diabetic mice (Sun et al., 2008). It was found that *I. obliquus* also enhanced the serum levels of insulin and alleviated the metabolic derangement of glucose enzymes in STZ-induced diabetic mice (Wang et al., 2017). Similarly, polysaccharide extract obtained from *I. obliquus* was reported to exert a hypoglycemic effect by inhibiting the action of α-glucosidase, which resulted in a delayed glucose absorption in the digestive organs following meals (Chen, Lu, Qu, Wang, & Zhang, 2010). An ethyl acetate extract from *I. obliquus* inhibited the activity of α-amylase and increased the serum level of high-density lipoprotein cholesterol in alloxan-induced diabetic mice (Lu, Chen, Dong, & Zhang, 2010). Treatment of diabetic nephropathy in C57BL/6 mice with polysaccharide extracts of *I. obliquus* elevated insulin levels; however, the cholesterol and triglyceride levels remained unaffected (Chou et al., 2016). After treating STZ-induced diabetic mice with an IOP-chromium (III) complex for 4 weeks, the fasting blood glucose levels, plasma insulin levels, and body weight were significantly decreased (Wang et al., 2017). The hypoglycemic and pancreatic β-cell regenerative effects of *I. obliquus* are shown in Figure 1, and the antidiabetic effect as well as possible molecular mechanisms are shown in Table 2.

TABLE 3 Possible anticancer molecular mechanisms of *Inonotus obliquus* in in vivo and in vitro studies

Extracting solvent or component of *I. obliquus* used	Experimental model	Dosage (animals) or concentration (cell line) used	Effects and possible molecular mechanism described	Reference
Hot water	Human hepatoma cell lines	750 and 100 μg/m	Induced apoptotic death of hepatoma cells in a dose-dependent manner by decreasing the expression levels of p53, cyclins D1, Cdk2, Cdk4, and Cdk6.	Youn et al., 2008
Hot water	BALB/c mice implanted with B16-F10 melanoma	20 mg/kg of animal weight for 10 days	Inhibited tumor growth, and arrested tumor cells at G0/G1 phase, reduced pRb, p53, and p27 expression levels.	Youn et al., 2009
Ethanol	human colon cancer (HT-29) cell line	2.5–10 μg/ml	Inhibited the proliferation of tumor cells by enhancing the expression levels of p21, p27, and p53, and suppressing the phosphorylation of Rb and E2F1.	Lee et al., 2015
Lyophilized powder	Human colorectal carcinoma (HCT-116) cell line	5–25 mg/ml	Inhibited the proliferation of colorectal carcinoma by decreasing the expression levels of CyclinD1,NF-κB, and enhancing the expression of proapoptotic genes (p53, p21WAF1/CIP1)	Tsai et al., 2017
Inotodiol	Human lung adenocarcinoma (A549) cell line	40 and 80 mg/L	Suppressed the proliferation of lung carcinoma cells at the S phase, and ↓the expression level of Ki-67, and Bcl-2 in a dose-dependent manner	Zhong et al., 2011
	Human gastric (BGC2823) cell line	80 mg	↑caspase-3 and Bax expression, ↓the expression levels of Bcl-2, and suppressed tumor growth	Zhong et al., 2010
Ergosterol peroxide	Human colorectal (HCT-116) cell line	5–20 μg/ml	Suppressed tumor growth and formation of clonogenic colony, and decreased the levels of β-catenin	Kang et al., 2015
Polysaccharides	Human nonsmall cell lung carcinoma (A549) cell line	10–100 μg/ml	Inhibited the metastasis and invasive ability of tumor cells by down-regulating the expression levels of COX-2, MMP-2 and MMP-9, and reducing the phosphorylation of MAPK and PI3K/AKT, in a dose-dependent manner.	Lee et al., 2014
Water	AOM/DSS mice, Human colorectal (DLDl and HCT116) cell line	100 or 300 mg/kg of animal weight, 0.2 and 0.5 mg/ml	Inhibited the proliferation of colon cancer cell by reducing the activities of Wnt/β-catenin and NF-κB pathways	Mishra et al., 2013

5 | INHIBITORY EFFECT OF *I. OBLIQUUS* EXTRACTS ON TUMOR GROWTH AND CELL CYCLE

Globally, cancer is among the leading causes of death, with an estimated 8.2 million cancer-related deaths being recorded in 2012, and the numbers of new cancer cases are projected to surge to 23.6 million by 2030 (https://www.cancer.gov/about-cancer/understanding/statistics6). Homeostasis in normal cells is achieved through critical processes, for example, cell proliferation and death; however, the distortion of this tightly regulated cellular process that arises as a consequence of the dysregulation of cell cycle mechanisms has been implicated in the pathogenic processes of most cancers (Senderowicz, 2003). In mammals, the progression of the cell cycle is tightly regulated by the cyclins, that is, cyclin-dependent kinases (CDKs), and CDK inhibitors. Each cellular cycle phase is controlled by distinct CDKs, which are regulated by their individual cyclins (Johnson & Walker, 1999). The overexpression of CDKs and cyclins has been implicated in the pathogenesis of most cancers (Hall & Peters, 1996). The metastasis of cancers entails an array of complex interconnected processes, and the expression of different proteins such as the matrix metalloproteinases (MMPs) family and cyclooxygenase-2 (COX-2) has been implicated in this process (Cui, Zhang, & Fu, 2008; Fidler, Kim, & Langley, 2007; Shiraga et al., 2002).

Arresting the cell cycle of dysfunctional proliferating cells offers a therapeutic approach for potential anticancer agents. Although most agents for chemotherapy of human cancers currently used can clinically halt the disease progress and inhibit/destroy tumors, the detrimental consequences associated with these agents on healthy cells and tissues remain a major concern (Sporn, 1999). These undesirable effects latter make the need for effective and safe chemotherapeutic agents increasingly important.

It was found that *I. obliquus* extract decreased the viability of human hepatoma cell lines (HepG2 cell line) in a dose-dependent manner, arrested cancer cells at the G0/G1 phase, and induced apoptotic cell death by reducing the expression levels of p53, cyclins D1, Cdk2, Cdk4, and Cdk6 (Youn et al., 2008). Similarly, in vivo studies in Balb/c mice implanted with B16-F10 melanoma cells reported that a 10-day intraperitoneal administration of aqueous extracts from *I. obliquus* at a dose of 20 mg·kg·day suppressed tumor growth by decreasing the expression of pRb, p53, and p27 and arresting the proliferation of tumor cells at the G0/G1-phase (Youn et al., 2009). A

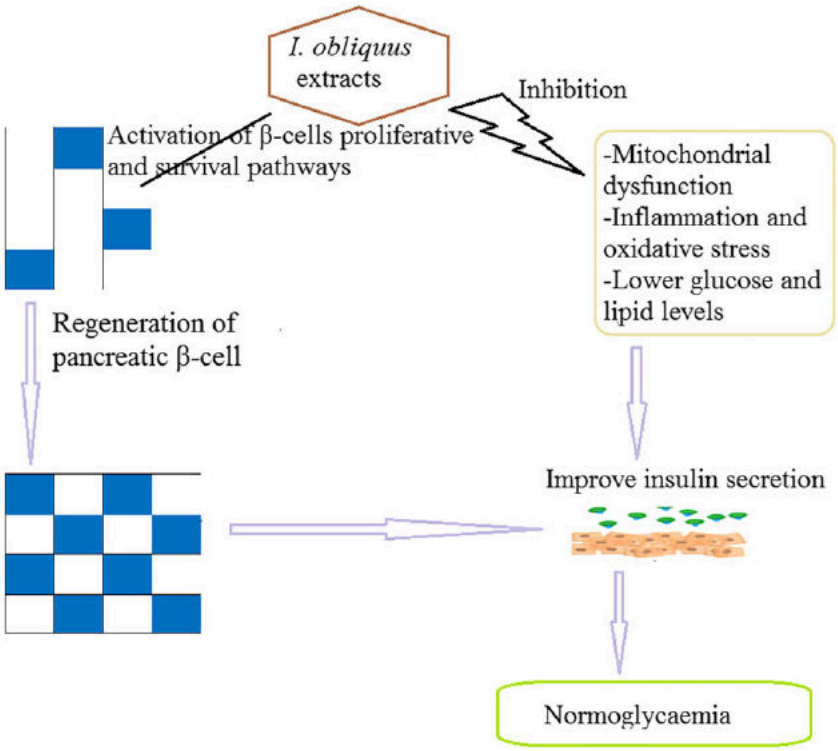

FIGURE 1 Mechanism of action of *Inonotus obliquus* on insulin secretion and the regenerative process of pancreatic β-cell (survival and proliferation). *I. obliquus* enhances the regeneration of the pancreatic β-cell after diabetic damage by activating various molecular pathways involved in the survival and proliferation of β-cell. Simultaneously, *I. obliquus* also inhibits hyperglycemia, mitochondrial dysfunction, inflammation, and oxidative stress, which lead to improved insulin secretion and normoglycemia. The blue box represents a pancreatic β-cell [Colour figure can be viewed at wileyonlinelibrary.com]

study in human a nonsmall cell lung carcinoma cell line (A549 cell line) also supported that polysaccharides obtained from *I. obliquus* inhibited the metastasis of the cancer cells by reducing the phosphorylation of MAPKs, PI3K, and AKT and decreasing the expression levels of MMP-2, MMP-9, COX-2, and NF-κB (Lee, Lee, Song, Ha, & Hong, 2014). Furthermore, the aqueous extracts of *I. obliquus* inhibited the proliferation of colorectal cancer (Mishra et al., 2013), and the treatment of AOM/DSS mice with the same aqueous extracts inhibited the growth of colon tumor (Mishra et al., 2013). Additionally, in vitro studies in HCT116, HT-29, SW620, and DLD-1 colorectal cancer cell lines showed that ergosterol peroxide from *I. obliquus* suppressed the growth and formation of a clonogenic colony of colorectal cancer cells by reducing the levels of β-catenin (Kang et al., 2015). Studies in the human colorectal carcinoma (HCT-116) cell line further revealed that the extracts obtained by submerged fermentation from *I. obliquus* enhanced the apoptosis of cancerous colorectal cells, increased the expression levels of p53, and suppressed NF-κB expression (Tsai, Li, & Lin, 2017). The aqueous extracts from this mushroom were found to preserve splenic lymphocytes from tumor-induced apoptosis (Chen, Zheng, Ga, & Xiang, 2007) and prevented the proliferation and metastasis of cancer cells (Burczyk, Gawron, Slotwinska, Smietana, & Terminska, 1996; Rzymowska, 1998). An in vitro study in human colon cancer cells (HT-29) further revealed that ethanolic extracts of *I. obliquus* reduced the levels of CDK2 and CDK4, increased the expression of p53, p21, and p27, and arrested the proliferation of the cancer cells at the G_1 phase (Lee, Kim, & Kim, 2015). Similarly, the exposure of human lung adenocarcinoma cell line (A549 cell line) to inotodiol extract from *I. obliquus* reduced the expression of Ki-67 and arrested the proliferation of the cells at the S phase in a dose-dependent manner (Zhong, Wang, & Sun, 2011). In addition, the exposure of human gastric BGC2823 cell line to 80 mg/L of *I. obliquus* extract enhanced caspase-3 and Bax expression and reduced the expression of Bcl-2 (Zhong, Sun, Gao, et al., 2010). It was reported that the endo-polysaccharide extract from *I. obliquus* enhanced the proliferation of Raw 264.7 murine macrophages suggesting that the anticancer properties of these carbohydrates are related to their immunostimulating effects rather than their tumoricidal activity (Kim et al., 2006). However, the cytotoxic evaluation of endo-polysaccharide from *I. obliquus* in different cancer cell lines revealed no cytotoxic effect of this extract neither in normal cells nor in most cancer cells even at high doses, with the exception of human hepatoma Hur7 and human breast adenocarcinoma MCF-7 cell line (Kim et al., 2006). It was found that although water-soluble polysaccharide extracted from *I. obliquus* suppressed growth of transplantable SGC-7901 cells in mice in a dose-dependent manner (Fan, Ding, Ai, & Deng, 2012), a contrasting finding of the same extracts was observed in a SGC-7901 cell line (Fan et al., 2012). Although the anticancer effect of *I. obliquus* appears to be significant, it needs to be clearly stated that more studies are required to better understand the anticancer molecular mechanisms of the components of this mushroom. The possible anticancer molecular mechanisms of action of *I. obliquus* are shown in Table 3.

6 | INFLUENCE OF *I. OBLIQUUS* EXTRACT ON THE IMMUNE SYSTEM

The undesirable consequences associated with classic cancer therapies, such as chemotherapy, radiotherapy, and surgical approaches, have instigated the need to develop other therapeutic approaches

with perhaps less adverse effects, for example, the use of immunotherapy (Khawar, Kim, & Kuh, 2015). Immunostimulation is a vital mechanism that the body employs to bolster its line of defenses, and enhancing this process in the treatment and management of cancer patients serves as an alternate therapeutic approach (Borchers, Krishnamurthy, Keen, Meyers, & Gershwin, 2008). The immunostimulation process requires an array of biochemical reactions, such as the activation of cell-mediated immunity via the secretion of interferon (IFN)-γ by Th1 cells and the stimulation of the humoral immunity via the secretion of IL-4 and IL-10 by Th2 cells. The balance between the cytokines produced by the Th1 and Th2 cells remains vital for proper immune response (Pinto, Arredondo, Bono, Gaggero, & Díaz, 2006).

It has been reported that polysaccharides isolated from the fruiting body of *I. obliquus* stimulated immune response in a dose-dependent manner by enhancing the secretion of IFN-γ/IL-4 and exhibiting comitogenic activity in ConA-stimulated splenocytes (Won et al., 2011). Kim et al. (2006) reported that intraperitoneal administration of endo-polysaccharide obtained from *I. obliquus* (at a dose of 30 mg·kg·day) to B16F10-implanted mice showed no cytotoxic effect on the tumor cells but rather enhanced the survival rate of the animals (4.07-fold) by its immunostimulating effect. A 24-day oral treatment regimen with aqueous extract from *I. obliquus* of chemically immunosuppressed mice enhanced the immune response by increasing the number of granulocytes-macrophage colonies and erythroid burst-forming units and up regulating the serum levels of IL-6 (Kim, 2005). Similarly, it was found that the oral administration of ovalbumin-sensitized BALB/c mice with hot water extract from *I. obliquus* at a dose of 100 mg/kg reduced serum IgE and IgG_{2a}, suppressed the production of IL-4, and enhanced the activity of IFN-γ in concanavalin A-stimulated splenocytes (Ko, Jin, & Pyo, 2011). A 4-week dietary treatment of the olive flounder fish, *Paralichythys olivaceus*, with *I. obliquus* extract improved the innate immunity of this marine species and conferred resistance against *Uronema marinum* parasites by enhancing the activity of lysozyme and complement and increasing the activity of myeloperoxidase (Harikrishnan, Balasundaram, & Heo, 2012). Additionally, oral treatment of chickens with *I. obliquus* fermentation products during vaccination improved peripheral blood mononuclear cells and the ratio of Th1/Th2 and suppressed the virulence of Newcastle disease virus (Zhang et al., 2018). Furthermore, in vitro studies in RAW 264.7 macrophages showed that lignin complexes from *I. obliquus* stimulated phagocytic activity, scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, and enhanced the production of NO (Niu et al., 2016).

6.1 | Antiinflammatory and antioxidative effects of *I. obliquus* extracts

Biological tissues constantly produce reactive oxygen species (ROS) that play vital roles in various cellular signaling pathways. The relationship between oxidative stress and metabolic disorders such as T2DM, cancer, obesity, and cardiovascular disease has been indicated by several studies (Fatehi-Hassanabad, Chan, & Furman, 2010; Rains & Jain, 2011). The distorted balance between the production and elimination of free radicals from the cell in favor of the former process gives rise to oxidative stress. The link between inflammation and cancer remains an undisputable fact; tumor cells tend to progress further in microenvironments rich in growth factors and inflammatory cells that promote their growth (Balkwill & Mantovani, 2001).

Yun et al. (2011) reported that *I. obliquus* extracts ameliorated oxidative stress, inhibited lipid peroxidation, and repressed premature senescence in human fibroblasts by suppressing the activities of MMP-1 and MMP-9 and enhancing the production of collagen in in vivo hairless mice. The freeze dried extracts of *I. obliquus* scavenged DPPH free radicals in a dose-dependent manner and exhibited a higher superoxide radical scavenging activity when compared with L-ascorbic acid (Cui et al., 2005).

Although the molecular mechanisms leading to insulin resistance are not fully understood, oxidative stress; the build-up of amyloid in the pancreas; accumulation of lipid in the muscle, liver, and pancreas; and endoplasmic reticulum stress are some of the key hypothesized mechanisms implicated in insulin resistance and the dysfunction of islet β-cells in T2DM. Although it remains difficult to disclose which mechanism is the most crucial in a T2DM model or an individual, it is interesting to note that each of these cellular stress factors either promotes an inflammatory response or is aggravated by inflammation (Ehses, Ellingsgaard, Boni-Schnetzler, & Donath, 2009; Masters et al., 2010). A hypothesis suggested that T2DM occurs due to the release of large amounts of cytokines from adipose tissue during an acute phase inflammatory reaction (Pickup & Crook, 1998). The link between hyperglycemia and the production of ROS has been highlighted by various studies, and it has been reported that hyperglycemia enhanced the generation of mitochondrial ROS by influencing the activity of the mitochondrial electron transfer chain (Lambert & Brand, 2004). Mitochondrial leakage of ROS promotes the creation and release of proinflammatory mediators (cytokines and chemokines), which have been implicated in both the manifestation of β-cell dysfunction and insulin resistance. The treatment of STZ-induced diabetic Wistar rats with polysaccharide extracts of *I. obliquus* for 6 weeks decreased IL-1β levels, retarded TNF-α production, and lowered the formation of ROS (Diao et al., 2014). Similarly, the treatment of H_2O_2-stimulated mice livers with extracts obtained from *I. obliquus* grown on germinated brown rice (*IOGBR*) enhanced both antioxidant enzymes activity and reduced malondialdehyde (MDA) levels (Debnath, Park, Kim, Jo, & Lim, 2013). These authors also reported that IOGBR extracts exhibited antiinflammatory effects in a lipopolysaccharide-stimulated RAW 264.7 cell line by suppressing proinflammatory mediators such as NO, PGE2, iNOS, COX-2, TNF-α, IL-1β, and IL-6 (Debnath et al., 2013). Ethanolic extract (70%) of *I. obliquus* retarded the phosphorylation of Akt, 1kBα, and MAPKs in lipopolysaccharide-activated macrophages in a dose-dependent manner (Kim et al., 2007). Three days of treatment of HFD/STZ-induced nephropathic mice with low molecular weight of polysaccharide extract of *I. obliquus* decreased the expression levels of NF-κB and transforming

growth factor and protected against STZ + advanced glycation end-products-induced glucotoxicity in renal tubular cells (LLC-PK1), in a dose-dependent manner (Chou et al., 2016). Other studies on rat liver tissue supported that IOP suppressed thiobarbituric acid reactive substance formation in Fe^{2+}/ascorbate-induced lipid peroxidation (Chen et al., 2010). Treatment of scopolamine-induced memory-deficient mice with a methanolic extract of *I. obliquus* decreased the nitrite, GSSG/GSH ratio, and MDA levels, whereas levels of antioxidant enzymes such as superoxide dismutase (SOD) and reduced glutathione (GSH) were increased (Giridharan, Thandavarayan, & Konishi, 2011). A study in PC12 cells reported that 3,4-dihydroxybenzalacetone isolated from *I. obliquus* inhibited oxidative damage in H_2O_2-induced oxidative stress (Nakajima, Nishida, Nakamura, & Konishi, 2009). The ethyl acetate extract of *I. obliquus* scavenged DPPH-free radicals, reduced the MDA level, and enhanced the GSH level in the liver tissue of alloxan-induced diabetic male Kunming mice (Lu et al., 2010). Recent studies reported that the treatment of T2DM male Kunming mice with IOP-chromium (III) complex reduced the MDA levels, and the activities of antioxidant enzymes in the liver (e.g., SOD, catalase, and GSH-Px) were upregulated (Wang et al., 2017). The antiinflammatory and antioxidative molecular mechanisms of *I. obliquus* are shown in Figure 2.

7 | THE ACTION OF *I. OBLIQUUS* ON ADIPOSE TISSUE DIFFERENTIATION

White adipose tissue has long been known as a crucial site for the storage of surplus energy derived from food intake and plays a prominent role in the metabolism of glucose and lipids. White adipocytes, the key cell type in white adipose tissue, acts as storage for dietary energy, which is stored as triglycerides. In times of starvation and energy deficit, these stored triglycerides can be converted to fatty acid (lipolysis), which are carried to other tissues for the production of energy through the mitochondrial fatty acids β-oxidation process. Studies in both mice and humans showed that adipose tissue dysfunctions are associated with increased circulating concentrations of fatty acids and triglycerides, resulting in insulin resistance (Shimomura et al., 1998; Sovik, Vestergaard, Trygstad, & Pedersen, 1996). The adipose tissue is critical for normal secretion of adipokines (e.g., leptin and adiponectin), which improve insulin sensitivity. Studies in human and animal models showed that lipodystrophies are associated with weakened adipokine secretion (Oral et al., 2002). Appropriate functional adipose tissue in proportion to the body size is paramount for normal glucose homeostasis and insulin sensitivity. The manifestation of a chronic inflammatory condition in the adipose tissue is coupled with the overexpression of cytokines such as TNF-α, particularly, by macrophage and sometimes the adipocytes. Overproduction of the TNF-α affects adipocyte functions (mostly the storage of triglycerides), impairs insulin signaling, and inhibits the adipogenesis process. Studies have reported that TNFα strongly reduced the expression of diverse specific adipocyte genes and adipogenic transcription factors, for example, that of C/EBPα and PPARγ (Zhang et al., 1996). TNFα can influence the expression PPARγ at different levels, such as the transcription, translation, and turnover of PPARγ mRNA and protein. It has been reported that although *I. obliquus* hot water extract activated adipogenesis of 3T3-L1 preadipocytes and enhanced the expression PPARγ target genes (adipocyte protein 2 aP2, lipoprotein lipase LPL, and CD36), additionally, the triacylglycerol accumulation was also improved (Joo, Kim, & Yun, 2010). Similarly, the treatment of Wistar rats with polysaccharide from *I. obliquus* lowered lipid peroxidation products (such as low-density lipoprotein) level, whereas high-density lipoprotein cholesterol level was enhanced (Diao et al., 2014). The possible molecular mechanisms exerted by *I. obliquus* on adipose tissue differentiation are shown in Figure 3.

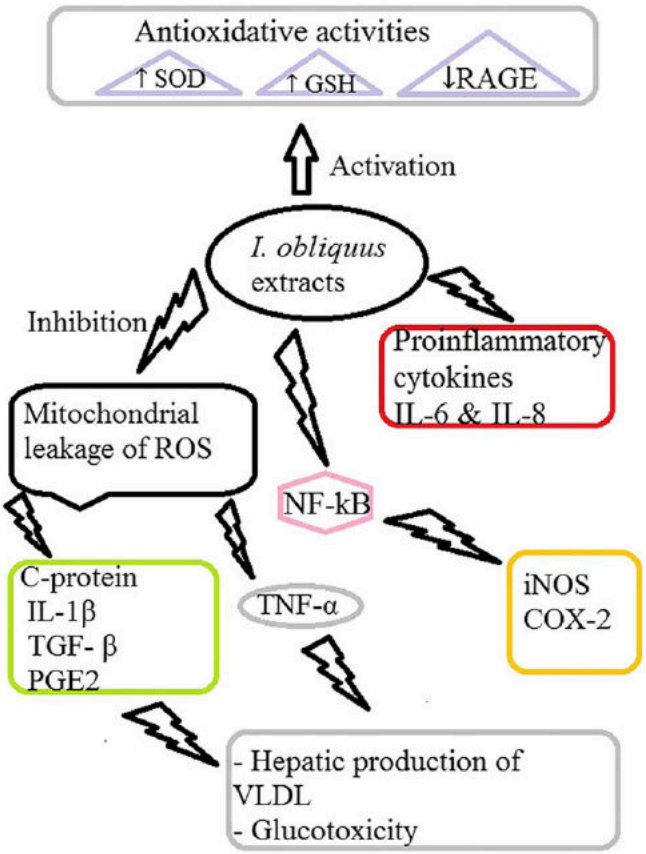

FIGURE 2 Antiinflammatory and antioxidative molecular mechanism of *Inonotus obliquus* exhibit antioxidant activities by enhancing the expression of superoxide dismutase (SOD) and reduced glutathione (GSH), while suppressing the expression of RAGE. The antiinflammatory mechanism of *I. obliquus* involves the inhibition of proinflammatory cytokines (such as IL-6, and IL-8) production. Also, *I. obliquus* inhibits the NF-κB pathway and suppresses the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). In addition, *I. obliquus* prevents the mitochondrial leakage of reactive oxygen species (ROS), which inhibits the expression of TNF-α, C-protein, IL-1β, TGF-β, and PGE2, which result in the decreased hepatic production of very low-density lipoprotein (VLDL) and reduced glucotoxicity [Colour figure can be viewed at wileyonlinelibrary.com]

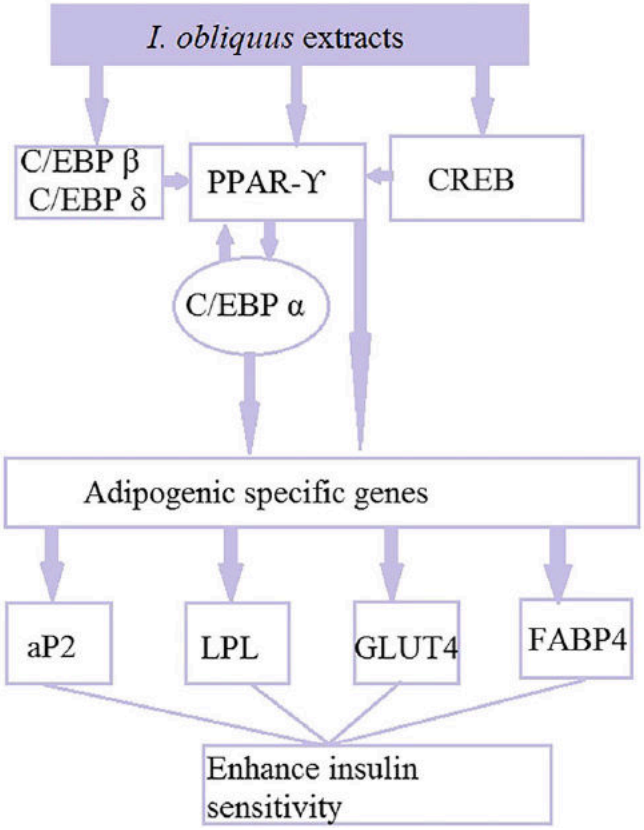

FIGURE 3 The molecular actions of *Inonotus obliquus* on adipose differentiation *I. obliquus* enhances the expression of (C/EBP) β and δ and CREB, which afterwards increase the expression of PPAR-γ. *I. obliquus* also activate the expression of PPAR-Υ directly. The expression of PPAR-γ lead to the upregulation of C/EBP α and the adipogenic specific genes, such as the adipocyte protein 2 (Ap2), lipoprotein lipase (LPL), glucose transporter 4(GLUT4), and fatty acid binding protein 4 (FABP4), which in turn, lead to improve insulin sensitivity [Colour figure can be viewed at wileyonlinelibrary.com]

8 | ANTIVIRAL AND OTHER THERAPEUTIC EFFECTS OF *I. OBLIQUUS* EXTRACTS

Several studies have reported on the antiviral effects of mushrooms and their constituents. It was observed that bioactive constituents of mushrooms could directly suppress viral enzymes and nucleic acids and indirectly enhance the immune system (Piraino & Brandt, 1999). Water extract from *I. obliquus* was shown to suppress the infectivity of hepatitis C virus within 10 min in porcine embryo kidney cells (Shibnev et al., 2011) and prevented the replication of HIV in a lymphoblastoid cell line at a concentration of 5.0 μg/ml (Shibnev, Garaev, Finogenova, Kalnina, & Nosik, 2015). Similarly, water soluble lignin obtained from this mushroom suppressed HIV-1 protease (Ichimura, Watanabe, & Maruyama, 1998).

The process of platelet aggregation involves a series of complex interconnected biochemical pathways. Initiation of normal hemostasis follows stepwise processes that involve the exposure of platelets to a subendothelial matrix followed by their subsequent adhesion to collagen through specific cell-surface receptors. The role of platelet aggregation and adhesion has been implicated in the pathogenesis of thrombosis (Diminno & Silver, 1983), and suppressing of platelet function has been highlighted as a promising therapeutic approach in the management of this clotting disorder. The treatment of male ICB mice with a tripeptide (~365 Dalton) purified from the mycelia of *I. obliquus* inhibited platelet aggregation, which leads to the possible use of this mushroom extract in the management of thrombosis (Hyun et al., 2006). Another possible therapeutic use of *I. obliquus* extract includes its ability to suppress asthma (Yan et al., 2011). Furthermore, daily treatment of carrageenin-induced paw edema in rats with ethanolic extract from *I. obliquus* at doses of 100 and 200 mg/kg decreased acute swelling, exhibited analgesic activity, and suppressed the expression of iNOS and COX-2 (Park et al., 2005).

9 | LIMITATIONS AND FUTURE RECOMMENDATION

I. obliquus has been shown to alleviate hyperglycemia, suppress tumor growth, enhance immune responses, and inhibit viral replication indicating the potential therapeutic relevance of this mushroom. Although a plethora of findings have supported its potential antidiabetic, anticancer, antiviral, and immunostimulating properties, it is worthy to note that results from animal and cell studies do not always correlate well with those obtained from human studies. In this respect, we suggest the need of a large number of well-controlled, long-term clinical studies of this mushroom as a potential anticancer, antiviral, and antidiabetic therapeutic agent. There is also a need to investigate whether the various foregoing therapeutic effects of *I. obliquus* are associated with any specific component(s) or are due to the combined effect of the numerous individual constituents. It is also necessary to determine whether the wide range individual components interact or antagonize the mode of action of each other. Although most scientific studies on the pharmacological potential of this mushroom as an antidiabetic agent have focused on the antiinflammatory, antioxidative, and pancreatic β-cell proliferation parameters, its effects on adipose tissue differentiation and leptin secretion have not been extensively studied.

Furthermore, it must be kept in mind that in the studies cited in this review, a wide variety of polar and nonpolar solvents and a plethora of extraction methods were used to obtain extracts from *I. obliquus* mushrooms (Table 1). Clearly, these different extraction protocols may have significantly affected the quality, quantity, and chemical composition of extracts. These, together with the biological variability in the mushrooms used (e.g., geographical location, climate, and soil conditions), storage techniques and possible contamination (e.g., environmental chemicals) make the source material inconstant. It is therefore conceivable that dissimilar components and in varying quantities were obtained and used in each study, making interstudy comparisons difficult. When human clinical studies are to be conducted, source material as well as extraction and isolation methods will need to be carefully standardized.

It has also been reported that there are different quantities and types of polysaccharides extracted from the natural sclerotia

and the cultured mycelia of *I. obliquus*, and their antioxidant properties vary. The polysaccharide extract from cultured mycelia *I. obliquus* showed a higher antioxidant effect when compared with the extract obtained from the natural sclerotia (Xu, Wu, & Chen, 2011). A comparison study of the IC_{50} of *I. obliquus* extracts obtained using water, ethanol, and methanol showed that the IC_{50} of methanol extracts was 24.90 mg/ml, whereas that of ethanol and water extracts were 16.25 and 18.96 mg/ml, respectively (Glamočlija et al., 2015). It was similarly reported that the time, temperature, and ultrasonic (frequency) influenced the antioxidative activity of polysaccharides extract from *I. obliquus* using the ultrasonic extraction method (Fu, Chen, Dong, Zhang, & Zhang, 2010). It is also important to note that various extraction protocols and the diversity of the *I. obliquus* specimens could also influence the therapeutic properties. In this respect, the antioxidant activities of extracts of *I. obliquus* obtained using three different solvents (phosphate-buffered saline, methanol, and ethanol) were compared, and it was found that despite detecting the highest total phenol content in phosphate-buffered saline extract, the 80% ethanol extract of *I. obliquus* exhibited the highest inhibitory effect on the activity of xanthine oxidase and free radical scavenging activities (Szychowski et al., 2018). Furthermore, it was observed that in normal fibroblasts (BJ cell line), ethanol extracts from *I. obliquus* enhanced the expression of SOD1 and catalase and reduced ROS production, whereas in cancer cell lines (Caco-2 cell line), an inverse effect of the extracts was observed (Szychowski et al., 2018). It was found that *I. obliquus* extract at doses >100 μg/ml exerted cytotoxic effects both to normal and cancer cell lines, and the bioactivity of *I. obliquus* extract was also found to vary depending on the route of administration (oral vs. intravenous injection; Song et al., 2007). Furthermore, *I. obliquus* extract was reported to both promote and suppress pulmonary colonization of CT-26 cell in BALB/c mice. At a dose of 100 mg/kg, a 35.9% increment in tumor metastasis was observed, whereas at a dose of 10 mg/kg, there was 33.4% inhibition of tumor metastasis (Song et al., 2007). Additionally, a promising antidiabetic therapeutic effect has also been found with vanadium-enriched *I. obliquus* (Zhang et al., 2011), which opens the door to the potential use of this mushroom in combination with metals and other bioactive compounds. It was further reported that the *I. obliquus* cultured in Tween 80 (nonionic surfactant)-containing medium improved the production of exopolysaccharides and endopolysaccharides and increased the scavenging activity against DPPH radicals (Xu, Quan, & Shen, 2015). Identifying potential therapeutic agents from natural sources with little to no toxicity, that could target different molecular pathways involved in the pathogenesis of cancer, DM, and viral replication, other than those affected by *I. obliquus*, would be of significant importance in harnessing the therapeutic use of *I. obliquus* products.

10 | CONCLUSION

Owing to the wide spectrum of potential health benefits associated with *I. obliquus*, many studies have been conducted to try to understand the molecular mechanisms of this mushroom in alleviating a number of metabolic diseases and cancer. Various experimental data have supported that extracts obtained from *I. obliquus* may possess anticancer, antidiabetic, antiviral, and immunostimulating properties. This is due to the constituents of these extracts being able to act as scavengers of ROS, reduce insulin resistance and inflammation, suppress tumor growth and metastasis, inhibit viral replication, and enhance immune responses. However, it is essential to emphasize that most experiments conducted on the therapeutic potentials of this mushroom are somewhat anecdotal and therefore lack the necessary scientific rigor and standardization. Although most studies, highlighting the pharmacological effects of *I. obliquus*, have been carried out in animals and cellular models, the need to conduct similar investigations of this mushroom in people is mandatory as studies in cell and animal models do not automatically accurately reflect results obtained in humans. More studies are needed to elucidate the antidiabetic, anticancer, antiviral, and immunostimulating activities of the individual components of *I. obliquus* and to reveal any possible synergistic/antagonistic effects between individual ingredients of this mushroom. Additionally, there is a need to identify chemical compounds, metals, and other potential naturally occurring therapeutic substances or existing therapeutically used agents that could act synergistically with *I. obliquus* in the treatment of DM and cancers.

ACKNOWLEDGEMENTS

This work was supported by the RSF (Grant 16-15-000390-П) and RFBR (Grants 17-03-00641 and 18-29-12129mk), and the Ministry of Education of the Russian Federation within the framework of the basic part of the state task, Project No. 4.9514.2017/8.9 and the Program 211 of the Government of the Russian Federation No. 02. A03.21.0006.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The scoping of relevant articles was done by P van der Bijl and I. G. Danilova, and the graphical design was done by I. G. Danilova and K. C. Duru. K. C. Duru, E. G. Kovaleva, and P van der Bijl drafted and revised the manuscript. The final manuscript was read and approved by all authors.

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How to cite this article: Duru KC, Kovaleva EG, Danilova IG, van der Bijl P. The pharmacological potential and possible molecular mechanisms of action of *Inonotus obliquus* from preclinical studies. *Phytotherapy Research*. 2019;33:1966–1980. https://doi.org/10.1002/ptr.6384