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Review Article

A Comprehensive Insight into the Effect of Berberine on Nonalcoholic Fatty Liver Disease (NAFLD): A Systematic Review

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Hepatic dysfunction is primarily caused by nonalcoholic fatty liver disease (NAFLD). Recently, berberine (BBR) has attracted researchers' interest with its hepatic protective property. A systematic review was conducted to evaluate the effects of BBR and its mechanisms of action in the management of NAFLD and its complications. The guidelines of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statements were applied to perform the study. Embase, Web of Science, Google Scholar, Science Direct, PubMed, ProQuest, and Scopus databases were searched up until March 2023. According to the inclusion criteria, finally, 65 studies were entered into the study. The evidence provided in the study revealed that BBR could regulate the development of NAFLD via several mechanisms of action namely lowering body weight, modulating lipid and glucose metabolism, and reducing inflammation and oxidative stress (OS). The current systematic review demonstrated the beneficial effects of BBR on NAFLD and its associated metabolic disorders including dyslipidemia, obesity, and insulin resistance through regulating lipid metabolism, facilitating β-oxidation of fatty acids, and mitigating the accumulation of triglycerides in hepatocytes. These beneficial effects make BBR a potential therapeutic approach and an efficient agent in the management of NAFLD and its related risk factors. There is an insufficient number of clinical trials addressing the effects of BBR in humans, so conducting more human research in the future is recommended.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), one of the most common health problems globally, is responsible for chronic liver diseases in both developing and developed countries [1, 2]. It is characterized by the abnormal accumulation of triglyceride (TG) and free fatty acids (FFAs) in hepatocytes (more than 5% of liver weight). In addition, NAFLD is known to be associated with metabolic comorbidities namely impaired glucose metabolism, obesity, hyperlipidemia, and insulin resistance (IR) [3]. The wide spectrum of NAFLD varies from simple steatosis to nonalcoholic steatohepatitis (NASH), necroinflammation, cirrhosis, and even hepatocellular carcinoma [3]. In accordance with an estimation, the prevalence of NAFLD is about 25.2% (nearly one billion adult individuals) worldwide [1]. It has been

demonstrated that Western dietary patterns, low physical activity, and genetic predisposition play an impressive role in the enlargement of NAFLD [4]. Regarding the hypothesis of the "two-hit," TG accumulation occurs in the first hit due to some factors namely IR, obesity, diets with higher fats, and absence of physical activity [5]. In the second hit, oxidative stress is caused by excessive fat accumulation, resulting in mitochondrial dysfunction, hepatic cell damage, and further liver cell injury [5].

Although numerous metabolic pathways have been suggested to initiate the development of NAFLD, the exact mechanism remains to be investigated [6]. It has been elucidated that NAFLD emerges from either elevated de novo lipogenesis of FFAs or lipolysis from adipose tissues and hepatic TG disposal. This lipolysis is through the oxidation or secretion of very-low-density lipoprotein (VLDL) particles [7]. Furthermore, insulin resistance and severe inflammation are both implicated in the development of NAFLD through enhancing the expression of lipogenic enzymes namely fatty acid synthetase (FAS) and acetyl-CoA carboxylase 1 (ACC-1) and inhibiting the expression of fatty acid oxidation enzymes including carnitine palmitoyl transferase 1a (CPT1a) [8, 9]. In addition, β-oxidation insufficiency and inflammatory signaling pathways, which stimulate oxidative stress (OS), could induce both IR and liver damage [10]. Several studies have demonstrated that natural compounds such as berberine, rutin, naringin, curcumin, quercetin, epicatechin, and resveratrol, along with healthy lifestyle modifications such as regular physical activity and a healthy diet, can ameliorate NAFLD [11].

Berberine (BBR), a quaternary ammonium salt is quinoline alkaloid with the chemical formula of $C_{20}H_{18}NO_4^+$, is a natural composite derived from several plants, especially *Berberis vulgaris L*, which is from the Berberidaceae family [12]. Previous studies have elucidated numerous pharmacology functions of BBR including antitumor, anticancer, antidiabetic, antioxidative, anti-inflammatory, cardioprotective, antiplatelet aggregation, enhancing immunological functions, decreasing blood lipid levels, Cerebro-protective, antimalaria, and antimicrobial [12]. It has been demonstrated that the hepatic tissues are the major target of BBR since the liver has the highest concentration of BBR metabolites (approximately 70 times as large as its concentration in serum) [13]. Recently, BBR has shown protective effects on hepatic damage, and it has been considered an effective treatment for NAFLD [13]. It has been well-documented that there is a close association between stress oxidative and fatty liver diseases. BBR could prevent reactive oxygen species (ROS) production by suppressing the expression of NADPH oxidase-2 (Nox-2), which is responsible for the cytoplasmic production of ROS [14]. Furthermore, BBR reduces the glucose levels of serum by upregulating the expression of glucose transporter 4 (GLUT4). In a study conducted on a high-fat diet (HFD)-induced NAFLD rat models, BBR ameliorated NAFLD through the regulation of inflammatory responses, which in turn prevented the release of proinflammatory cytokines such as TNF-α and IL-6 [15]. Given the findings of several studies, BBR could exert protective effects on NAFLD and its related complications and could prevent liver damage [12, 15]. Nowadays, there are several drugs and commercial products containing BBR such as *coptis chinesis*, so it is crucial to assess the potential effects of BBR [16]. Moreover, to our knowledge, no systematic review has ever summarized results on this topic. Therefore, the present study was designed as a comprehensive systematic review of published studies to evaluate the therapeutic effects of this natural compound on NAFLD and its consequent risk factors by elaborating upon the possible mechanisms of BBR's function.

2. Methods

2.1. Search Approach. The current systematic review was performed concerning the guidelines of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statements (Table 1, Electronic Supplementary Material). The study was registered in the PROSPERO (CRD42021277602). A comprehensive search was conducted in Embase, Web of Science, Science Direct, Google Scholar, PubMed, ProQuest, and Scopus, to identify relevant studies up to March 2023. The merger of MeSH and non-MeSH terms were as follows: "berberine" [Mesh], "fatty liver" [Title/Abstract], "nonalcoholic fatty liver disease" [Mesh], "NAFLD" [Mesh], "liver fibrosis" [Title/Abstract], "NASH" [Mesh], "hepatic steatosis" [Title/Abstract], "nonalcoholic steatohepatitis" [Title/Abstract], "insulin resistance" [Title/Abstract], "lipid and glucose metabolism" [Title/Abstract], "diabetes" [Title/Abstract], "T2DM" [Title/Abstract], "oxidative stress" [Mesh], "inflammation" [Mesh], "obesity" [Mesh], "BMI" [Title/Abstract], "fat mass" [Title/Abstract], "dyslipidemia" [Mesh], and "free fatty acids [Title/Abstract]." The search methods in the databases have been supplementary files. The selected studies were limited to English-language articles and those which were published up until March 2023. Moreover, we manually checked all reference lists of eligible studies in order to avoid missing any relevant studies. Finally, all recorded studies found by manual or electronic searches were entered into EndNote software (EndNote X8, Thomson Reuters, New York) for screening.

2.2. Inclusion Criteria. The inclusion criteria of the current study involved the following: (a) available in English-language studies, which were relevant to the current systematic review topic, (b) all clinical trials, animal, and *in vitro* studies that explored the impacts of BBR supplementation on NAFLD and its consequent disorders, and (c) no extra supplementation must be administered with BBR.

2.3. Exclusion Criteria. The exclusion criteria were based on the following: (a) non-English Language articles, (b) studies with inadequate data, (c) studies that assessed the effects of BBR on diseases other than NAFLD, and studies that examined the effects of a mixture of natural compounds on NAFLD, and (d) review studies, comments, presentation, report, editorial, or books chapters.

Table 1: Characteristics of studies that reported the potential roles of berberine on NAFLD.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
In vitro studies					
[17]	*In vitro*	HepG2 and AML12 cells exposed to high glucose and palmitic acid	20 μM	24 h	Reduction in hepatic TG accumulation and the expression of hepatic SCD1 and other TG synthesis-related genes; promotion of the phosphorylation of AMPK and SREBP-1c
[18]	*In vitro*	QSG-7701 cells	10 or 20 mM	24 h	Inhibition of the expression levels of ROS, MDA, TNF-α, NLRP3, NLRP3, caspase 1, GSDMD, TXNIP, SLC27A1, α-SMA, TGF-β1, FABP1, CYP2E1, ATF-4, CHOP PPAR-γ TLR4, p-NF-κB/NF-κB, and p-I-κB/I-κB; increase in the expression levels of PPAR-α and ACOX1
[19]	*In vitro*	L02 cell	0, 5, and 15 μM	24 h	Decrease in the mRNA levels of genes related to lipogeneses such as NLRP3, CYP2E1, ATF-4, and CHOP in L02 cell; increase in the expression levels of AMPK; decrease in MDA and LDH in L02 cell; no effect on the phosphorylation AMPKin H4IIE cells
[20]	*In vitro*	HepG2 cells induced by oleic acid (OA)	0, 0.0, 0.1, and 1 μM	24 h	Decrease in the fat overload and TG content in OA-induced HepG2 cells; increase in the mRNA of FXR; reduction in the mRNA of SREBP-1c and FAS in a dose-dependent manner
[21]	*In vitro*	MIHA and HepG2 cells	0, 10, and 20 μM	24 h	Upregulating the EGR1 level which functioned to transactivate miR-373 expression in MIHA and HepG2 cells. Subsequently, miR-373 depleted its target gene AKT serine/threonine kinase 1 (AKT1) mRNA level, which led to the inhibition of the AKT-mTOR-S6K signaling pathway in hepatocytes that were critical in the development of hepatosteatosis
[22]	*In vitro*	QSG-7701 cells	0, 10, and 20 μM	24 h	Decrease in the phosphorylation of ABCA1 serine residues and PKCd Tyr 311
[23]	*In vitro*	H4IIE cells	10, 25, and 50 μM	24 h	Reduction in the phosphorylation state of JNK1 in hepatoma H4IIE cells, the mRNA levels of genes related to lipogeneses such as ACC, FAS, CPT1a, jnk1, and SREBP1c; increase in the expression levels of AMPK; decrease in IL-6, IL-1β, and TNF-α in H4IIE cells BBR did not significantly increase the phosphorylation AMPKin H4IIE cells
[24]	*In vitro*	Human hepatoma cell line, HepG2	0, 1, 10, and 25 μM	24 h	Activation of AMPK induced the phosphorylation of extracellular-signal-regulated kinases 1/2 (ERK1/2) and subsequently induced CCAAT/enhancer-binding protein β (C/EBPβ) binding to the C/EBP-response element in the CD36 promoter in hepatocytes

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[25]	*In vitro*	HepG2 and FAO	5 μM	24 h	Suppression of the mRNA expression of phospho-PERK, phospho-eIF2α, ATF6, and SREBP-1c; reduction in cellular TG in oleate acid/palmitate acid (OA/PA)-induced excessive lipid accumulation in cultured HepG2, FAO, and primary hepatocytes
[26]	*In vitro*	HepG2 cell	1, 5, 10 μM	24 h	BBR significantly decreases TC SREBP2 and 3-hydroxy 3-methylglutaryl-CoA reductase levels BBR significantly increases mRNA and protein levels of SIRT1, as well as higher acetyl-FoxO1 protein level compared to the FFA-only group
[27]	*In vitro*	Huh7	10 μM	24 h	Reduction in the expression of MRAK052686 and Nrf2 was completely reversed by BBR treatment, suggesting a new mechanism accounting for the therapeutic effect of BBR
[28]	*In vitro*	RAW264.7	10 μM	24 h	Inflammasome caspase 1, pannexin-1. Limiting the activation of the purinergic receptor P2X7, involved in the late phases of NLRP3 (NACHT, LRR, and PYD domain-containing protein 3) inflammasome. Upon P2X7 knockdown, the ability of BRB to block LPS-induced secretion of IL-1β was lost
[14]	*In vitro*	Huh7 and HepG2	10 μM	24 h	Suppression of the expression levels of Nox2, complex I, II, and III, the expression levels of Nrf-2, HO-1, and SOD; decrease in mitochondrial-derived ROS production induced by FFA
[29]	*In vitro*	HepG2	—	24 h	No significant effect on Mn-SOD, and UCP2 expressions
[30]	*In vitro*	HepG2	10 μM	24 h	No significant effect on mRNA expression levels of iNOS
[31]	*In vitro*	HepG2	10 or 20 mM	24 h	Activating AMPK by increasing 3 its phosphorylation
[32]	*In vitro*	BAT-SVF cells	0.25 or 0.5 mM	24 h	Elevating the transcription of PRDM16, a master regulator of brown/beige adipogenesis, by inducing the active DNA demethylation of PRDM16 the promoter, which might be driven by the activation of AMPK and production of its downstream tricarboxylic acid cycle intermediate α-Ketoglutarate; increase in the expression levels of UCP1, PRDM16, and PPAR-γ
[33]	*In vitro*	RAW264.7	0–10 μM	24 h	Inhibition of the expression of both mRNA and protein levels of proinflammatory cytokines (TNF-α, IL-6, IL-1β, MCP-1) and ER stress genes (CHOP, ATF4, and XBP-1) significantly; inhibition of PA/LPS-induced inflammatory responses through modulating ER stress-mediated ERK1/2 activation in macrophages and hepatocytes

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[34]	*In vitro*	HepG2	1.25, 2.5, and 5 μM	24 h	Berberine treatment similarly prevented lipid accumulation by regulating the protein expression of ATGL, GK, PPAR-α, CPT-1, ACC1, FAS, and CD36
In vivo studies					
[12]	*In vivo*	Rats ($N = 16$)	100 and 300 mg/kg/day/HFD	8 weeks	BBR-treated rats had reduced liver wet weight, improved liver steatosis, and a significant decrease in liver TG levels, while ALT, AST, TC, TG, and LDL serum levels significantly decreased and MTTP levels were significantly upregulated. In conclusion, BBR treatment ameliorated the fatty liver induced by a high-fat diet in rats. Furthermore, BBR reversed the abnormal expression of MTTP and LDLR in rats with high-fat diet induced-NAFLD
[35]	*In vivo*	Rats/high-fat diet ($N = 8$)	300 mg/kg/day/oral	8 weeks	Improving liver histopathology and serum proinflammatory cytokines (TNF-α, IL-6) and free fatty acid (FFA) levels; amelioration nonalcoholic steatohepatitis through, at least partly, restoring the Treg/Th17 ratio; regulating the chemerin/CMKLR1 signaling pathway to reduce liver inflammation and reducing lipid deposition
[36]	*In vivo*	Rats ($N = 16$)	100 mg/kg/day IP	16 weeks	Reduction in both body and liver weight, TG, TC, ALT, AST, HDL-C, and LDL-C; increase in the protein expression levels of SIRT3, p-AMPK, p-ACC, and CPT-1A in the liver; attenuating liver injury with the HFD
[17]	*In vivo*	Mice ($N = 10$)/normal chow diet	3 00 mg/kg/day oral	4 weeks	Reduction in hepatic TG accumulation and the expression of hepatic SCD1 and other TG synthesis-related genes; attenuating hepatic steatosis through the activation of AMPKSREBP 1c-SCD1 pathway
[37]	*In vivo*	Rats ($N = 10$) high-fat diet	300 mg/kg	4 weeks	Alleviating HFD-induced suppression of fatty acid β-OX through, at least partly, SIRT3-mediated LCAD deacetylation; decrease in the TG, TC, LDL levels, and body weight; increase in the HDL levels and insulin sensitivity; suppression of the expression of lipogenesis genes including SREBP-1c, SCD1, and FAS FAO; increase in the transcriptional expression of PPARa and CPT-1a
[8]	*In vivo*	Rats ($N = 20$) high-fat diet	250 mg/kg/d	8 weeks	Increase in the expression of AMPK; reduction in the TG, TC, AST, and ALT levels; inhibition of the expression of TNF-α, IL-6, CCL19, TLR4, NF-κB-p65, SREBP-1c, and FAS; improving HFD-induced steatosis

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[38]	*In vivo*	Rats/high-fat diet ($N = 11$)	150 mg/kg/oral	12 weeks	Decrease in firmicutes and cyanobacteria; reduction in gut permeability and improvement of the intestinal barrier in NAFLD rats
[39]	*In vivo*	Rats ($N = 20$) high-fat diet	150 mg/kg/d	16 weeks	Reduction in weight loss, lipid profiles, and HOMA-IR; elevating ISI; reduction in rates of glucose appearance, GNG, and hepatic lipogenesis; an increasing trend in the rate of fatty acid β oxidation in skeletal muscle; attenuating the ectopic liver fat accumulation
[25]	*In vitro*	Mice ($N = 7$)/MCD diet	200 mg/kg/d	5 weeks	Reduction in the levels of TG, TC, LDL, TG, BUN creatinine, AST, and ALT; inhibition of the accumulation of hepatic lipoperoxides; down-regulation of the expression of the mRNA levels of SREBP-1c, CHREBP, FAS, (TGF) β, α-SMA, CYP2E1, CYP4A10, and C/EBPβ; suppression of the expression of TNF-α and IL-6 in the liver; increase in the CAT; reduction in the mRNA expression of ATF6, XBP1, ATF4, P-PERK, P-EIF2α, and CHOP; alleviating liver steatosis confirmed by biochemical analysis of the hepatic triglyceride content; decrease in the TG, TC, AST, ALT, and TBARS levels
[40]	*In vivo*	Mice ($N = 10$)/HFHC	0.2 g/kg	12 week	Berberine had a strong antifibrotic effect, which was displayed by Masson's trichrome staining and hydroxyproline measurement Reduction in body weight, serum total cholesterol, triglyceride levels, liver index, steatosis score, serum ALT, and AST levels; suppression of NE expression in liver tissues; upregulation of α1-AT levels; decrease in liver mRNA levels of CXCR4, CXCL12, the mRNA expression of RAS, PLC-β, PI3K, AKT, NF-κB, IL-1, and IL-8
[13]	*In vivo*	Mice ($N = 10$)/normal chow diet	300 mg/kg/day	8 weeks	Improvement of liver histopathology, serum proinflammatory cytokines, and free fatty acid (FFA) levels; reduction in the mRNA expression of Angptl2, NF-κB, Foxo1 in the liver, the expression of CCL2, TNF-α, and CCR2 in the liver
[41]	*In vivo*	Rats ($N = 6$)/HF diet	150 mg/kg/oral	6 weeks	Upregulation of the mRNA expression level of AdipoR2, GLUT4; decrease in the AST, ALT, TC, TG, FFA, LDL, insulin, glucose, MDA, TNF-α, body weight, and liver weight; increase in levels of HDL, SOD, and adiponectin; suppression of the expression of pho-ERK/ERK

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[42]	*In vivo*	Rats ($N = 32$) high-fat diet	25, 50 and 100 mg/kg oxyberberine 100 mg/kg/ berberine oral	8 weeks	Alleviating inflammation via downregulating the mRNA expression of MCP-1, Cd68, Nos2, Cd11c, and enhancing Arg1 mRNA expression in white adipose tissue with oxyberberine and berberine treatment; increase in the expression of AMPK; an inhibition of aberrant phosphorylation of IRS-1; upregulating the downstream protein expression and phosphorylation (PI3K, p-Akt/Akt, and p-GSK-3β/GSK-3β) to improve hepatic insulin signal transduction with the administration of both oxyberberine and berberine treatment
[43]	*In vivo*	Rats ($N = 10$) high-fat diet	10 mg/100 g body weight	10 weeks	No effect on the levels of γ-GT, TC, HDL, LDL, and IL-10 in rats; decrease in the levels of, ALT, AST, TG; suppression of IL-17, IFNγ, TNF-α, IL-8, and IL-6; increase in the levels of TGF-β and body weight
[44]	*In vivo*	Mice ($N = 7$)	10 mg/kg/oral high-fat diet	7 days	Upregulation of the expression of hepatic CD36 and triglyceride levels in normal diet-fed mice
[45]	*In vivo*	Rats ($N = 8$)/oral high-fat diet	300 mg/kg/oral high-fat diet	6 weeks	Reduction in serum ALT, AST, TG, FFA, and glucose; increase in hepatic levels of glycogen; inhibition of the expression of SREAP-1c, FAS, TLR-4, TRL-9, NLRP3, and ASC in the liver; reduction in the expression levels of IL-1β, TNF-α, IL-8, and IL-6 in serum and liver; no effect on the expression levels of PPAR-α
[46]	*In vivo*	Rats ($N = 12$) oral high-fat diet	200 mg/kg/oral high-fat diet	12 weeks	Alleviating hepatic steatosis and inflammatory cell infiltration; reduction in the NAFLD activity scores, the NAS scores, serum levels of ALT, AST, TC, LDL-C, the levels of TNF-α, IL-6, and IL-1β, the expression of TLR4, MyD88, and NF-κB in the liver tissues; reversing the nuclear translocation of NF-κB in the primary liver cells
[47]	*In vivo*	Mice ($N = 11$) oral high-fat diet	200 mg/kg/oral high-fat diet	5 weeks	Inhibition of the expression of IL-1, TNF-α, IL-6, CD-14, the expression levels of IL-1β, TNF-α, IL-8, and IL-6 in serum and liver; restoring the gut microbiota; decrease in body weight, the levels of TG, TC, LDL, FBG, insulin, HOMA-IR, NAS, steatosis score, AST, and ALT
[48]	*In vivo*	Mice ($N = 10$)	aa/oral HFHC	12 weeks	Reduction in the levels of TC, LDL, glucose, AST, and ALT; increase in HDL levels; decrease in body weight and liver weight, the expression levels of FASN, SCD1, SREBP1c, IL-6, IL-1β, CD68, F4/80, MCP-1, TNF-α, the expression levels of angiogenic factors such as CD31 and VEGFR; suppression of phosphorylation of p38MAPK and ERK as well as COX2 expression

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[49]	*In vivo*	Rats ($N = 10$)	25 mg/kg/oral HFD	16 weeks	Restoring the expression of L-PK by the demethylation of L-PK promoter; increase in acetylation levels of histone H3K18, H3K9, H4K8, and H4K12 around L-PK; decrease in body weight, visceral fat, and liver weight
[50]	*In vivo*	Rats ($N = 10$)	100 mg/kg/oral HFD	8 weeks	Inhibition of the expression levels of the SREBP-1c, pERK, NF-κB, TNF-α, and pJNK; increase in the expression of caveolin-1, the HDL, and GPX levels; decrease in the TG, TC, LDL, ALT, AST, ALP, FSG, FSI levels, body weight, and visceral fat
[23]	*In vivo*	Rats ($N = 10$)	100 mg/kg/oral HFD	12 weeks	Reduction in inflammation in both the liver and adipose tissue as indicated by the reduction of the phosphorylation state of JNK1 and the mRNA levels of proinflammatory cytokines; decrease in hepatic steatosis, as well as the expression of acetyl-CoA carboxylase and fatty acid synthase; no effect on the phosphorylation state of AMPK in both the liver and adipose tissue of HFD-fed mice; improving systemic insulin sensitivity and glucose homeostasis; decrease in liver mRNA levels of IL-1β and TNF-α; no effect on liver mRNA levels of IL-6; amelioration of obesity-associated hepatic steatosis involves in a decrease in liver lipogenesis; decrease in the mRNA levels of genes related to lipogeneses such as ACC, FAS, CPT1a, and SREBP1c
[51]	*In vivo*	Rats ($N = 10$)	100 mg/kg/oral HFD	4 weeks	Reduction in liver TG contents, the FIN levels; no effect on the serum ALT, AST TG, TC, LDL, HDL levels, FBG levels, and IR protein levels; increase in GIR; upregulation of IRS-2 mRNA expression in high fat-diet fed rat livers with berberine or pioglitazone treatments for 4 weeks
[19]	*In vivo*	Mice ($N = 16$)	50 mg/kg and 150 mg/kg/oral HFD	4 weeks	Inhibition of the expression of mRNA levels of FASN, Fabp1 SLC27A1, α-SMA, TGF-β1, FABP1, CYP2E1, ATF-4, CHOP TLR4, p-NF-κB/NF-κB, and p-I-κB/I-κB; increase in the expression levels of PPAR-α, GSH, and ACOX1 in mice Decrease in serum levels of MDA, TNF-α, IL-1β, IL-6, the levels of liver TG, liver TC, LDL, FBG, insulin, HOMA-IR, NAS, ALT, AST steatosis score, and body weight

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[22]	*In vivo*	Mice ($N = 16$)	200 mg/kg/oral MCD	4 weeks	Alleviating hepatic lipid accumulation, plasma lipid levels, hepatic lipid deposition, ALT, and AST activities; reduction in steatosis by increasing ABCA1 protein levels through PKCd to reduce the phosphorylation of serine residues in ABCA1
[18]	*In vivo*	Mice ($N = 5$)	100 mg/kg/oral MCD	4 weeks	Decrease in lipid accumulation; ameliorating ROS and lipid peroxides, TNF-a expression, and phosphorylation of NF-κB p65
[31]	*In vivo*	Mice ($N = 16$)	20 and 40 mg/kg/oral HFD	4 weeks	Reduction in serum levels of MDA, TG, TC, MDA, ALT; increase in GSH in mice, the expression levels of AMPK, ACO, MCAD, ABCG5, and MTTP; downregulating the expression of the mRNA levels of SREBP1c, SREBP2, TNF-α, and IL-1β by demethylene berberine
[52]	*In vivo*	Rats ($N = 16$)	100 mg/kg/oral HFD	4 weeks	Decrease in serum levels of insulin, HbA1c, leptin, adiponectin, TG, phospholipids, albumin, and ALP; increase in HDL in mice; no effect on creatinine, total bilirubin, creatine kinase, acid phosphatase, LDH, AST, ALT, γ-glutamyl transferase, free fatty acids, atherogenic index; downregulating the expression of ROS; improving hepatic mitochondrial function possibly via activation of SirT3
[38]	*In vivo*	Rats ($N = 11$)	150 mg/kg/oral HFD	16 weeks	Reduction in body weight, gut permeability, and the variability of intestinal flora; improvement of the intestinal barrier in NAFLD rats
[53]	*In vivo*	Rats ($N = 6$)	Single-dose/HFD	48 h	Mitigation in serum levels of ALT, AST, and TG; no effect on the levels of epididymal fat, brown fat, liver weight, body weight, HDL-c, and TC
[32]	*In vivo*	Rats ($N = 10$)	1.5 mg/kg/day/oral HFD	8 weeks	Facilitating brown adipocyte differentiation; increase in the transcription of PRDM16, a master regulator of brown/beige adipogenesis, by inducing the active DNA demethylation of PRDM16 promoter, which might be driven by the activation of AMPK and production of its downstream tricarboxylic acid cycle intermediate α-Ketoglutarate; no impact on the BAT thermogenesis in adipose-specific AMPKa1 and AMPKa2 knockout mice
[54]	*In vivo*	Rats ($N = 10$)	162 and 324 mg/kg/day/oral HFD	12 weeks	Downregulating the expression levels of mRNA and UCP2; reduction in the levels of TG, TC, and LDL; increase in HDL levels
[14]	*In vivo*	Mice ($N = 10$)	200 mg/kg/oral HFD	16 weeks	Reduction in triglyceride accumulation in the liver of HFD-fed mice, Nox2-dependent cytoplasmic ROS production, and mitochondrial ROS production

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[55]	*In vivo*	Mice ($N = 10$)	50 mg/kg/oral HFS	6 weeks	Downregulating the expression levels of FFAs G6PDH and G3PDH; upregulating the expression levels of PPAR-γ; decrease in serum levels of IR, LDL, TC, and TG; increase in HDL levels
[28]	*In vivo*	Mice ($N = 10$)	50 mg/kg/oral HFS	28 days	Decrease in the expression levels of all components of the NLRP3 (NACHT, LRR, and PYD domain-containing protein 3) inflammasome caspase 1, pannexin-1, and hepatic levels of mature IL-1β, TNF-α, ALT; increase in GSSH levels; decrease in macrophages by preincubation with berberine
[13]	In vivo	Rats ($N = 8$)	300 mg/day high-fat diet comprising 21.6% protein, 36.1% fat, 42.3% carbohydrate; 82.75% basal diet + 10% lard, 2% cholesterol, 0.25% bile salt, and 5% egg yolk powder	12 weeks	Reduction in proinflammatory cytokines (CCL2, TNF-α); increase in the infiltration of inflammatory cells in the liver; suppression of the expression mRNA and protein levels of Angptl2, NF-κB, and foxo1 on different degrees
[27]	*In vivo*	Rats ($N = 10$)	50 mg/kg/oral HFD (32.6% carbohydrate, 51.0% fat, and 16.4% protein)	16 weeks	Reduction in the levels of TC and LDL in the liver
[56]	*In vivo*	Rats ($n = 6$)	200 mg/kg/oral HFD (32.6% carbohydrate, 51.0% fat, and 16.4% protein)	48 h	Reduction in the levels of ALT, AST, TG, TC, and LDL and increase in the levels of HDL Also, berberine significantly changes the expression level of liMTTP; CPT-1α, GCK; LDLR, L-PK
[12]	*In vivo*	Rats ($N = 23$)	50 mg/kg/oral HFD (composed of 80% regular chow, 8% yolk powder, 10% lard oil, 1.5% cholesterol, and 0.5% bile salt)	16 weeks	Decrease in body weight, hepatic wet weight, NAS score, serum levels of TC, TG, MTTP, FBG, ApoB, and LDLR; increase in HDL levels
[51]	*In vivo*	Rats ($N = 10$)	187.5 mg/kg/day/HFD	4 weeks	Berberine may improve insulin resistance of NAFLD by upregulating mRNA and protein levels of IRS-2
[34]		Mice ($N = 24$)	10, 20, 40 mg/kg	4 weeks	Berberine treatments could significantly improve hepatic steatosis and insulin resistance in high-fat diet (HFD)-fed mice BBR, could maintain glucose homeostasis via GLUT2, GSK3β, and G6Pase in HFD-fed mice
Human studies					
[32]	Clinical trails	Human ($N = 20$ patients)	0.5 g	1 month	Decrease in BMI, HOMA-IR, and body weight; increase in HDL levels; no effect on the serum levels of insulin and FBG
[53]	Clinical trials	Human ($N = 80$ patients)	0.5 g	16 weeks	Reduction in serum levels of APO-A, APO-B, TC, TG, BMI, and body weight; no effect on the levels of γ-GT, ALT, ASTAPO-E, LDL, Hb A1C, HOMA-IR, and serum insulin; increase in HDL levels
[56]	Clinical trials	Human ($N = 55$ patients)	0.5 g	16 weeks	Berberine significant reduction in body weight, AST, APO-E TG. TC, HOMA-IR but did not change in γ-GT, ALT, LDL, HDL, APO-A

Table 1: Continued.

Reference	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Main results
[57]	Clinical trials	Human ($N = 67$ patients)	500 and 100 mg	18 weeks	Berberine significantly decreases HbA1c, fasting insulin, fasting plasma glucose, LDL-C, ALT, AST, GGT, BMI, and liver fat content
[58]	Clinical trials	Human ($N = 50$ patients)	6.25 g	7 weeks	Berberine had no significant impact on FBS, ALP, fasting SGOT, SGPT, TG, TC, HDL LDL, ALP, BMI

THP1: human monocytic cell line, HepG2: hepatocellular cancer cell lines, TNF-α: tumor necrosis factor-α, IL- 6: interleukin 6, AST: aspartate aminotransferase, NO: nitric oxide, LPS: lipopolysaccharides, PI3K/AKT; phosphatidylinositol 3-kinase/protein kinase B, Nrf2: the nuclear factor erythroid 2-related factor 2, MCF-7: Michigan Cancer Foundation-7, MDA-MB-231: triple-negative breast cancer cell line, IL-1β: interleukin-1β, GSH: reduced glutathione, SMAD3: mothers against decapentaplegic homolog 3, MAPK: mitogen-activated protein kinases, GalN: d-galactosamine, FPG: fasting plasma glucose test, NO: nitric oxide, Nrf2: the nuclear factor erythroid 2-related factor 2, GSH: reduced glutathione, GR: glutathione reductase, MDA: malondialdehyde, CAT: catalase, TBARS: thiobarbituric acid reactive substances, NF-κB: nuclear factor-κB, JNK: Jun N-terminal kinase, MAPK: p38 mitogen-activated protein kinase, TG: triglycerides. TC: total cholesterol, HbA1c: hemoglobin A1C, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

2.4. Data Extraction and Quality Assessment. Eligible articles were retrieved using title/abstract by two independent authors, which consequently were assessed based on inclusion criteria. After the exclusion of irrelevant studies, the full text of remained articles was analyzed meticulously for eligibility and extraction of data. The extracted data from each study contained the first author, study location, study design, the subject of the study, duration and follow-up, year of publication, a dose of BBR, and the main conclusion. In cases of disagreement between reviewers, the controversial articles were discussed by researchers and resolved accordingly.

2.5. Risk of Bias Assessment. Two independent researchers (M.V. and P.A.) assessed the risk of bias for selected studies. Furthermore, the Cochrane risk of bias (ROB) was used to evaluate the overall degree of bias for randomized controlled trials. Regarding animal studies, the SYRCLE risk of bias tool was carried out to assess the overall risk of bias. The SYRCLE risk [59] of the bias tool comprises seven domains namely attrition bias, performance bias, detection bias, random sequence generation, reporting bias, allocation concealment, and other bias sources. The quality of the *in vitro* studies was assessed using the OHAT risk of bias tool [60]. These tools assessed attrition, detection, selection, reporting biases, and performance. It was given a "high risk" score for studies that contained methodological issues liable to affect their results, a "low risk" score for those without methodological issues, and an "unclear risk" score for those with inadequate data.

3. Results and Discussion

3.1. Literature Search. In order to find relevant English studies up to the end of March 2023, two authors independently searched databases. We identified 819 articles from databases (35 from Cochrane, 204 from Scopus, 134 from Embase, 78 from PubMed, 147 from Science Direct, and 221 from the Web of Sciences). The title and abstract of 321 articles remained after eliminating duplicate studies. The topic of the study led to the consideration of 125 studies.) An evaluation of 65 articles found 19 in vitro studies, 41 animal studies, and 5 human studies suitable for inclusion in the present study was conducted. The stages of the study are shown in Figure 1.

3.2. Risk of Bias. The OHAT risk of bias tool was applied to assess the *In vitro* studies. According to the qualitative assessment, most studies were rated as low risk of bias for the incomplete analysis, similarity of experimental conditions, confidence in the adequate administration of dose or exposure level, exposure characterization, and other bias sources. Almost all of these studies blinding of outcome evaluator were not noted clearly. Methods of allocation of groups were properly described in 65% of the included studies (Figure 2). All 41 animal studies were assessed for risk of bias using SYRCLE's tool. The qualitative assessment indicated that most studies were rated as low risk of bias for the group similarities at sequence generation category, other sources of bias category, and baseline category, and in most of these studies, blinding of outcome assessor, random outcome assessment, randomization in animal housing, and investigators/caregivers were not reported clearly. Methods of allocation concealment were properly reported in 75% of the included studies. The risk of biosselective outcome reporting and incomplete outcome data was identified in six (15%) studies and five (12%) studies, respectively (Figure 3). All five studies were randomized, and all of them reported the method of randomization. Methods of allocation concealment were properly described in four of the five included studies. One study was judged to be at high risk of bias which was prone to detection bias. The risk of bias was unclear for the remaining four studies (Figure 4).

This review paper properly addressed the effects of BBR on inflammation, lipid profile, glycemic parameters, obesity, and OS in both human and animal studies. The findings of the current systematic review demonstrated that BBR could attenuate NAFLD and its linked metabolic conditions namely obesity, hyperlipidemia, and insulin resistance. Both animal and human studies have confirmed that BBR can mitigate the inflammatory pathways namely NOD, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome and Toll-like receptor 4 (TLR4)/nuclear factor kappa-light-chain (NF-κB) signaling pathways, and stress oxidative by inhibiting the overproduction of ROS [46, 61]. Additionally, nearly all of the studies have shown the potential role of BBR in reducing serum lipids such as TC, LDL-C, TG, and increasing HDL levels [56]. Although, in some studies, berberine did not affect the serum levels of HDL, LDL, TG, TC, and hepatic enzymes, the majority of experiments indicated the beneficial effects of berberine on lipid profile [39, 62]. Of note, berberine can improve glucose metabolism by reducing insulin resistance, fasting blood glucose, and stimulating the uptake of glucose by peripheral tissues. The impact of BBR on body weight, as important comorbidity, was noticeable [51, 63]. In this regard, most studies confirmed the weight-lowering property of berberine [64].

3.3. Beneficial Effects of BBR on NAFLD and Its Related Complications and Mechanisms of Its Action. The mechanisms regarding the BBR function in the management of NAFLD and its related disorders are discussed in three sections obesity, metabolic risk factors, inflammatory parameters, and oxidative stress. The proposed multiple pathways for berberine action are presented in Figures 5 and 6.

3.3.1. Antiobesity Effects of Berberine. There is a positive association between the prevalence of NAFLD and body mass index (BMI) [65]. Therefore, dietary supplements and medications, which are prescribed for weight-lowering purposes, could be administered for the management of NAFLD patients [39, 56]. It is becoming evident that the treatment of obesity, at least slow weight loss, could result in

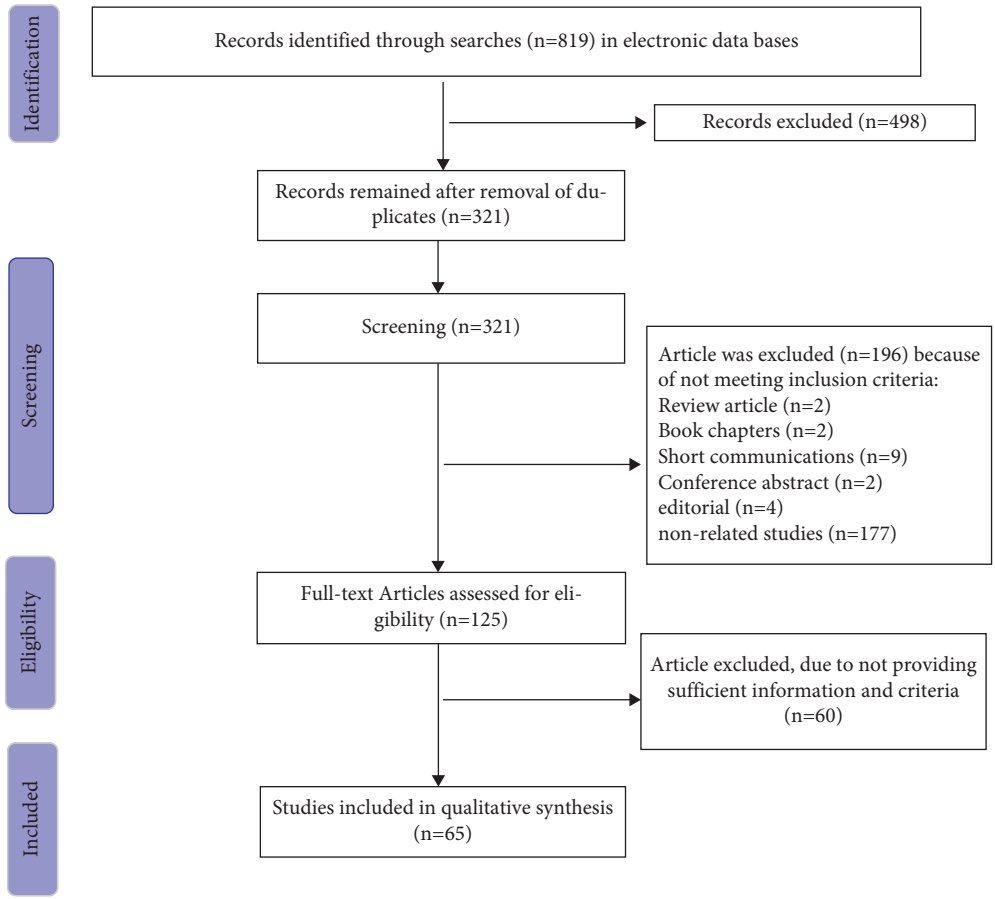
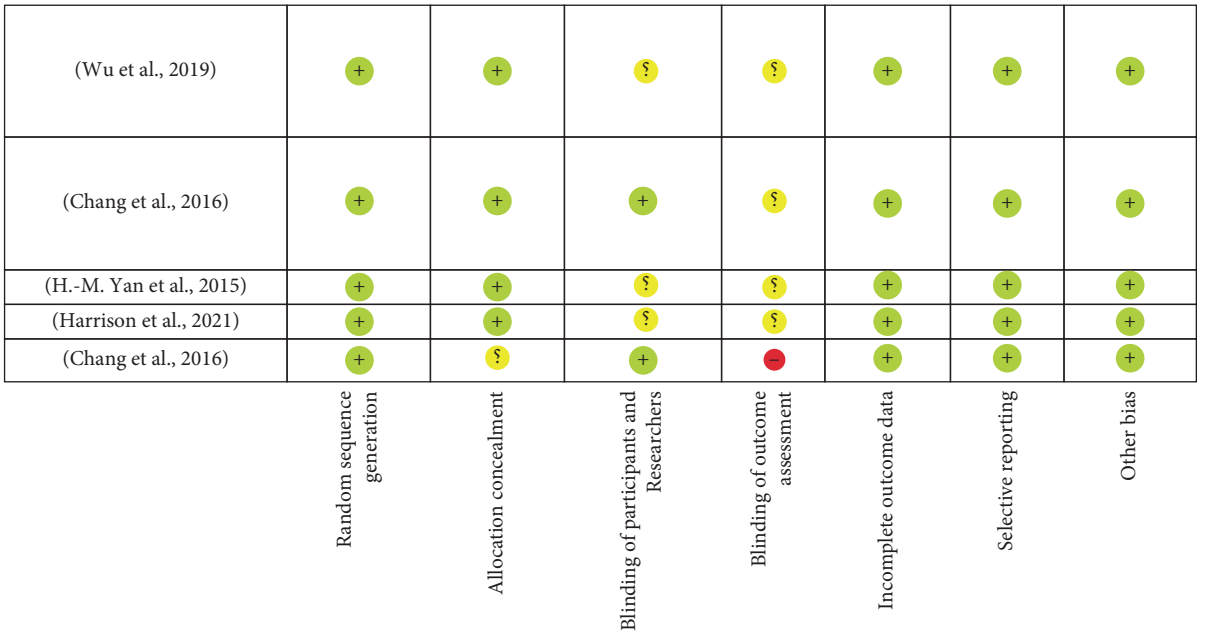


Figure 1: Flowchart of the process for selecting studies for the systematic review.

the improvement of liver fibrosis and inflammation, inhibition of fat accumulation, and mitigation of hepatic enzymes [66]. *In vitro*, *In vivo*, and human studies have shown that BBR could ameliorate obesity by different mechanisms, for instance, by preventing the expression of genes, which induce the differentiation and propagation of adipocytes, gut microbiota regulation, liver gluconeogenesis, and intestinal permeability [27, 39, 66]. BBR could enhance BAT mass and function, which in turn leads to the amelioration of obesity. Furthermore, in mice models, BBR increased the thermogenesis of BAT and total energy expenditure through BAT proliferation and expression of brown adipogenic genes [32]. BBR could induce the expression of liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) [18]. PPARs act as molecular sensors of fatty acids and control the metabolism of energy. It also has been indicated that BBR could increase the transcription of AMP-activated protein kinase (AMPK)-P PR domain-containing 16 (RDM16), a regularization of brown/beige adipogenesis, through modulating the active DNA demethylation of PRDM16 the promoter [18, 19, 32]. This might be due to the upregulation of AMPK, and downstream tricarboxylic acid cycle intermediates α-ketoglutarate. Furthermore, BBR could elevate the expression levels of uncoupling protein 1 (UCP1), PRDM16, PPAR-γ in BAT-SVF cells, which decrease FFA [18, 19, 32]. BBR significantly inhibited the expression of activating transcription factor 4 (ATF4), cytochrome P450 2E1 (CYP2E1), and C/EBP homologous protein (CHOP), which are involved in the process of oxidative stress and fatty acid β-oxidation, fatty acid uptake in the live and adipose tissue. Convincing evidence exerted that dysbiosis of the gut microbiota might induce metabolic inflammation via triggering metabolic endotoxemia [47]. It has been shown that BBR could improve obesity and insulin resistance by modulating gut microbiota [47]. In this regard, BBR induces the expression of insulin receptor substrate-1 (IRS-1) and insulin receptor (IRc), which consequently improves insulin resistance and obesity [42]. Another possible mechanism regarding the antiobesity property of BBR is through modulating gene expression, and it has been suggested that BBR suppresses the differentiation of adipocytes via controlling adipogenesis-mediated genes namely PPAR-γ, cAMP-response element-binding (CREB) protein, GATA-2, and GATA-3 [67, 68]. On the other hand, BBR could stimulate thermogenic genes in both BAT and weight adipose tissue (WAT), and UCP1 [32]. Previous studies reported that BBR could exhibit weight-lowering effects by inhibiting the fibrosis of adipose tissue [23]. BBR inhibits polarization and macrophage infiltration in the adipose



Each domain was scored as "−" if it contained methodological flaws that may have affected the results, "+" if the flaw was deemed inconsequential, and "?" if information was insufficient to determine. If a study got "+" for all domains, it considered as a high quality study with totally low risk of bias.

Figure 2: Results of risk of bias assessment for human studies.

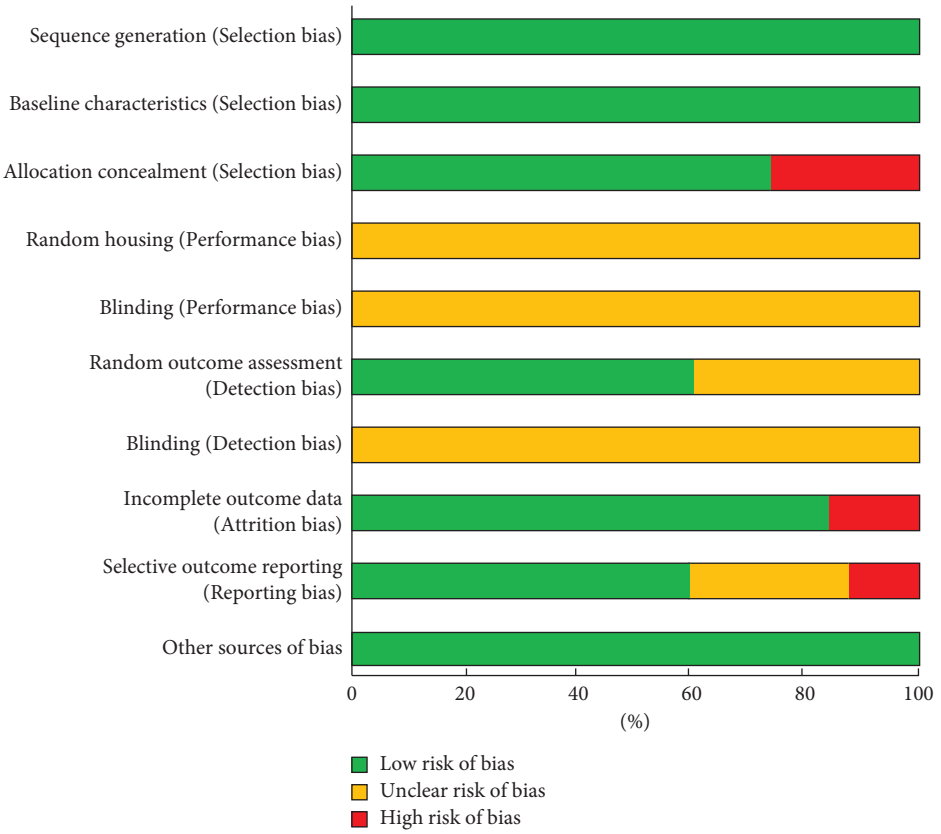


Figure 3: Results of risk of bias assessment for animal studies included in the current systematic review.

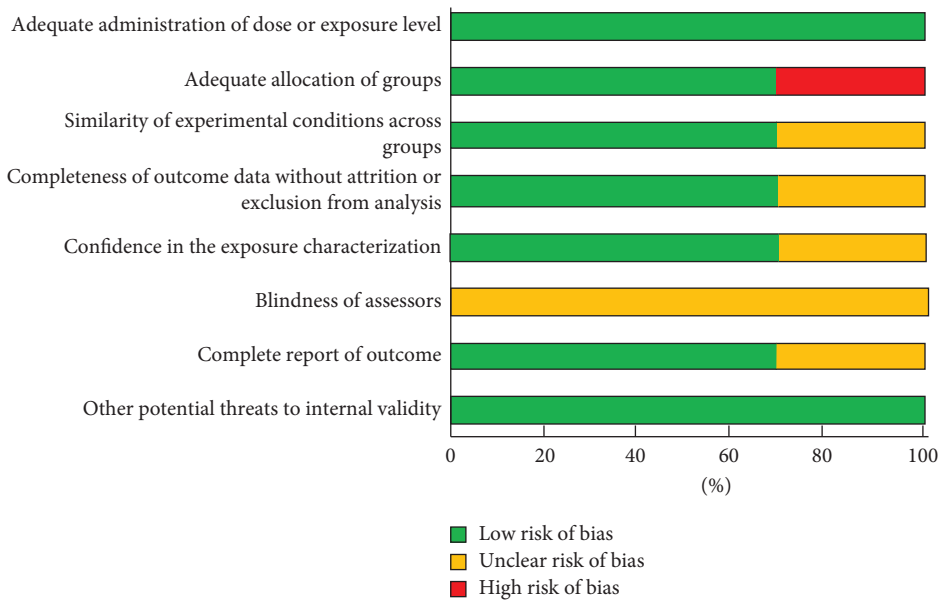


Figure 4: Results of risk of bias assessment for *in vitro* studies included in the current systematic review.

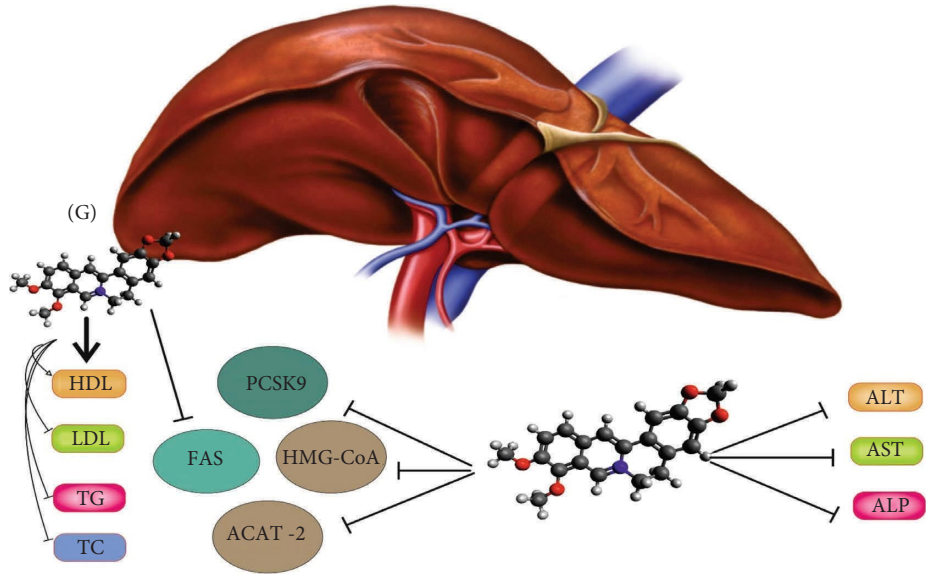


Figure 5: Aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). A cholesterol acyltransferase (ACAT), AMPK, 5′-adenosine monophosphate-activated protein kinase; proprotein convertase subtilisin/kexin type 9 (PCSK9), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), and increasing high-density lipoprotein (HDL).

tissue. In addition, in WAT, BBR could activate AMP-activated protein kinase (AMPK) and suppress the transforming growth factor (TGF)-β (TGF-β1)/Smad3 signaling pathway, which consequently alleviates adipose tissue fibrosis. Therefore, based on the aforementioned findings, it can be concluded that treatment with BBR attenuates obesity, as a vital risk factor for the pathogenesis of NAFLD, and its associated complications.

3.3.2. Effects of Berberine on Metabolic Risk Factors regarding NAFLD

(1) Lipid Metabolism. Compelling evidence has shown that BBR could play a vital role in numerous aspects of lipid hemostasis through different mechanisms. BBR ameliorates cholesterol levels by stimulating the expression of LDL receptors in hepatocytes, which is mediated through the

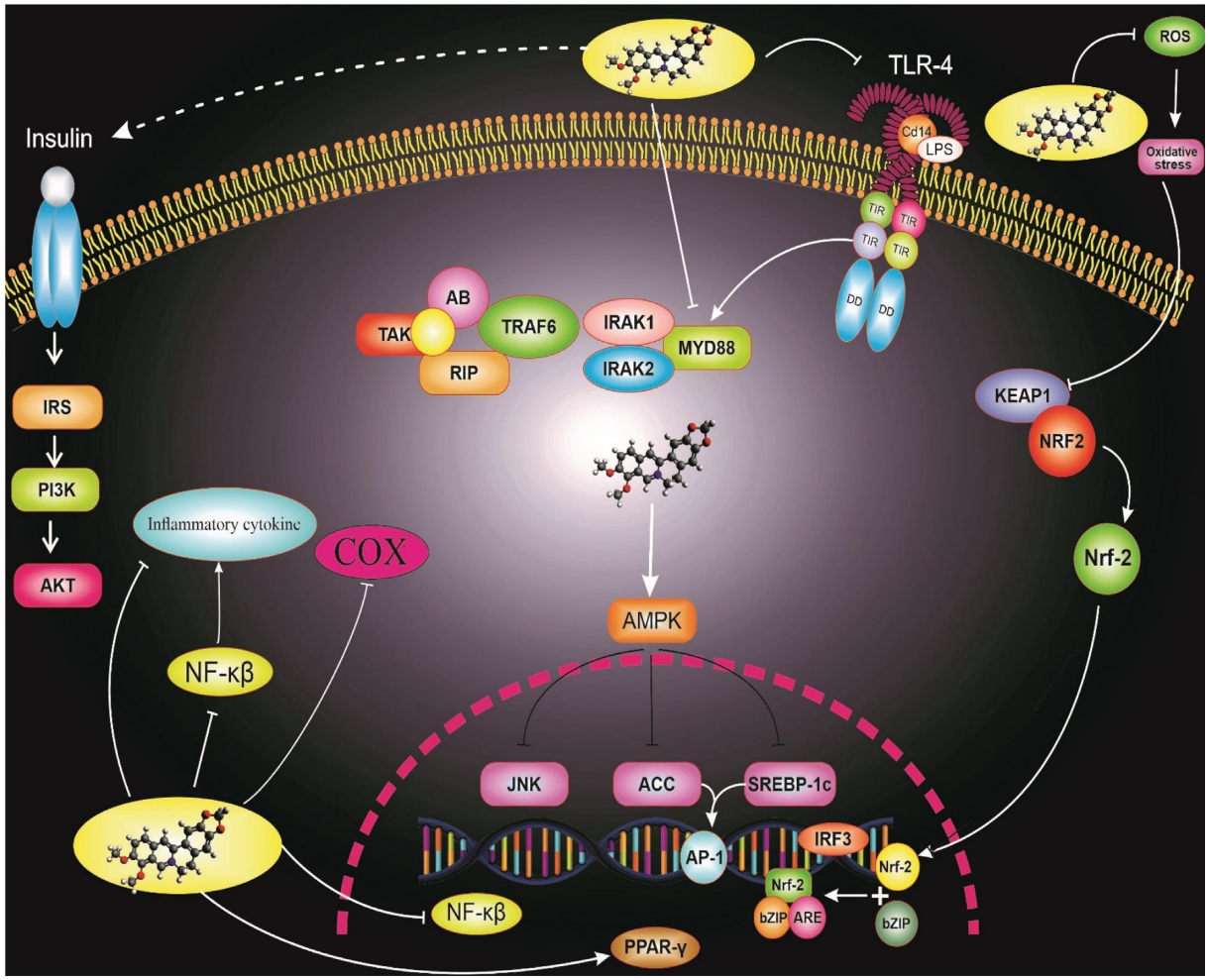


Figure 6: The cellular and molecular mechanisms of the potential roles of berberine on NAFLD. Berberine reduces the expression of inflammatory factors by preventing the expression of NF-κB and TLR-4 pathways. Berberine activates transcription factor Nrf-2 expression and increases nuclear translocation and subsequent antioxidant-responsive element (ARE) binding; therefore, protecting cells against oxidative stress damage by increasing the expression of antioxidant enzymes.

extracellular-signal-regulated kinase (ERK) pathway [25, 41]. Moreover, it has been documented that BBR induces the expression of LDL receptors through both suppressing the proprotein convertase subtilisin/kexin type 9 (PCSK9) LDL receptor pathway and downregulation of hepatocyte nuclear factor 1a (HNF-1a) protein, as a mediator for PCSK9 gene transcription [12, 69, 70]. PCSK9 increases the degradation of LDL receptors by diverting these receptors toward lysosomes [12]. Thus, the cholesterol-lowering effects of BBR might be due to suppressing the PCSK9 pathway [69, 70]. Another possible mechanism of BBR is through triggering the excretion of cholesterol from the liver into the bile, which ultimately leads to a reduction in cholesterol levels [36, 37]. Of note, BBR attenuates dietary cholesterol uptake through the alteration of intestinal absorption in Caco-2 cells by suppressing the expression of acyl-coenzyme A cholesterol acyltransferase (ACAT)-2 and inhibiting micellization of cholesterol in the intestinal lumen [71, 72]. Furthermore, in *in vitro* studies, BBR reduces intestinal permeability via Caco-2 tight junction monolayer. It is becoming evident that BBR promotes bile acid synthesis from cholesterol by stimulating the expression of mitochondrial sterol 27-hydroxylase and activation of cholesterol 7 alpha-hydroxylase [73, 74]. BBR also could promote fatty acid oxidation and inhibit lipogenesis via AMPK. The 3-hydroxy-3 methyl glutaryl-coenzyme A (HMG)-CoA reductase, a regulatory enzyme in the process of hepatic cholesterol synthesis, could be suppressed by BBR, which in turn reduces the cholesterol levels [8, 17]. The activation of AMPK by BBR inhibits SREBP-1c, which in turn attenuates NAFLD development. Xu et al. [37] reported that supplementation with 300 mg/day resveratrol for 4 weeks resulted in a significant decrease in serum levels of high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG). Also, they showed that BBR leads to suppression of the expression of lipogenesis genes including SCD1, FAS, FAO, sterol regulatory element-binding protein 1 (SREBP-1c), increase in the transcriptional expression of PPARa and CPT-1a in NAFLD rats. In other study, Zhao et al. [8] reported that BBR intake at a dose of 250 mg/kg body weight resulted in a reduction in serum TG and TC levels in NAFLD

rats, and they also suggested that BBR might reduce lipid profile by decreasing the expression of protein SREBP-1c and FAS.

(2) Glycemic Parameters. A growing body of evidence demonstrated the glucose-lowering effects of BBR in both clinical trials and animal studies. BBR has been shown to enhance p-IRS levels and ameliorate plasma insulin levels and glucose homeostasis via downregulation of c-Jun N-terminal kinase (JNK) signaling activity, P38 A mitogen-activated protein kinase (MAPK), and upregulation of AMPK [23, 75, 76]. BBR could improve the uptake of glucose by muscle cells via activating glucose transporter type 4 (GLUT-4) translocation, which is consistent with the upregulation of AMPK, an energy sensor that participates in the cellular metabolism by its regulatory activity [23, 75]. Also, the activation of the AMPK can modulate glucose metabolism in liver cells by inhibiting genes responsible for carbohydrate transport solute carrier family 2 member 1 (Slc2a1) and Slc2a4 and glucose metabolisms such as fructose-bisphosphate 1 (FBP1), glucose-6-phosphatase catalytic subunit (G6PC), glucose-6-phosphate dehydrogenase, glycerol-3-phosphate dehydrogenase 1 (GPD1), phosphoenolpyruvate carboxykinase 2, and mitochondrial PCK2, and glycogen synthase kinase 3 beta (GSK3B) [77, 78]. Of interesting, berberine induces Ser/Thr phosphorylation of IRS-1 and increases the phosphorylation of Akt via enhancing the expression of PI3K, which leads to the improvement of hepatic insulin resistance [42]. On the other hand, BBR improves IR by the PPAR-γ pathway [45, 55]. BBR inhibits PTP1B expression by increasing the activity of PKB, PI3K, and GLUT4 and decreasing the expression of GSK-3β and glucose 6-phosphate (G6P). Therefore, it increases the glycogen storage of the liver and reduced insulin resistance [79].

The PPAR and different isoforms of PPARs, particularly PPAR-γ, affect insulin metabolism and decrease the serum cholesterol, which acts as a vital ligand-dependent mediator in the homeostasis enzymes' expression [80]. BBR could also prohibit the aberrant phosphorylation of IRS-1 and upregulate the downstream protein expression and phosphorylation (p-Akt/Akt, PI3K, and GSK-3β/p-GSK-3β), which in turn leads to the improvement of hepatic insulin signal transduction [42]. In addition, berberine exerted protective effects against hepatic steatosis through the modulation of gut microbe, elevating the serum glucagon-like peptide-1 and neuropeptide Y, and attenuating orexin-A levels, which in turn regulates energy metabolism and reduces the animals' capacity to uptaking energy from food [36]. BBR has been shown to improve insulin resistance by stimulating the expression of insulin receptor substrate-2 (IRS-2) mRNA, a vital agent in the insulin signaling pathway, in hepatocytes [51]. Of note, BBR decreased the expression of metabolism-related genes like CPT-1a (regulates the oxidation of fatty acids), MTTP (regulates VLDL and LDL levels), and GCK responsible for the modulation of glucose metabolism rate [22]. Teodoro et al. [52] reported that different doses of BBR intake at a dose of 100 mg/kg b.w. improve hepatic insulin signal transduction with the administration of BBR. Orally supplementation of BBR for 4 weeks contributed to the decrease in serum levels of insulin, HbA1c. In another study, 187.5 mg/kg/day BBR for four weeks improved insulin resistance via upregulating mRNA and protein levels of IRS-2 [51]. Thus, it could be concluded that BBR might be a potential therapeutic agent in the treatment of NAFLD due to its beneficial effects on glucose metabolism [56].

(3) Hepatoprotective Effect of Berberine. Several studies indicated that BBR could reduce the serum level of liver enzymes namely ALT and AST, which in turn attenuates liver injury and steatosis [8, 41]. Serum levels of transaminases are the markers of liver steatosis and tissue impairment [81]. Therefore, one of the hepatoprotective effects of BBR might be through the modulation of liver enzymes [41]. For instance, in a study conducted on HFD rat models, 48 hours of BBR supplementation significantly reduced the serum levels of AST and ALT [56]. In other study, Chen et al. [12] investigated the effects of BBR on liver function in experimental NAFLD rats. Rats were orally administered with BBR at a dosage of 100 and 300 mg/kg/day/for 8 weeks. BBR treatment considerably reduced alanine transaminase (ALT) and aspartate transaminase (AST) levels in the liver. In one of the studies, the dose of BBR used varied from 50 to 150 mg/kg/b.w for 4 weeks, resulting in a considerable improvement in the levels of ALT, AST in NAFLD rats [19]. Moreover, 4 weeks of BBR administration at the dosage of 200 mg/kg/day in methionine- and choline-deficient (MCD) mice decreased the plasma levels of hepatic enzymes [22]. BBR could reduce liver damage by decreasing the regulation of NADPH oxidase-2 (NOX2).

3.3.3. Protective Effects of BBR on Inflammation and Stress Oxidative in NAFLD. It has been demonstrated that oxidative stress, mitochondria impairment, and inflammatory signaling pathways could also contribute to the pathogenesis of NAFLD [10]. According to the existing evidence, the serum levels of inflammatory markers are higher in NAFLD patients, while anti-inflammatory mediators have low concentrations. In this regard, BBR could alleviate the NAFLD progression by inhibiting Toll-like receptor 4 (TLR4)/nuclear factor-kappa*b* (NF-κB) signaling pathway [82]. The activated NF-κB, as a result of TLR 4-induced cascade of downstream signals, could lead to the activation of proinflammatory cytokines including IL-6 and TNF-α [8]. Proinflammatory cytokines, including TNF-α, play a vital role in the development of NAFLD by disrupting the insulin signaling pathway resulting in insulin resistance (IR) [82]. In addition, the upregulatory impacts of BBR on AMPK reduce the proinflammatory responses. Yan et al. [45] investigated the effects of oral supplementation of 300 mg of BBR for 6 weeks in HFD-induced NAFLD rats. The findings demonstrated that BBR could prohibit the expression of SREAP-1c, FAS, TLR-4, TRL-9, NLRP3, and ASC in the liver and reduce the expression of IL-1β, TNF-α, IL-8, and IL-6 in the liver; however, it did not affect the expression of PPAR-α. Regarding oxidative stress, BBR supplementation (100 mg/kg) for 4 weeks in 5 mice has been shown to suppress the

production of ROS, the expression of TNF-α, and the phosphorylation of NF-κB p65 [18]. Mai et al. [18] evaluated the effects of BBR on inflammation-related pathways in QSG-7701 cells. The results showed that BBR supplementation (10 or 20 mM) to QSG-7701 cells resulted in a reduction in the expression of TNF-α and an increase in the expression of PPAR-α and ACOX1. In other study, Wang et al. [33] investigated the effects of BBR (0–10 μM) in RAW 264.7 cells for 24 hours. They observed that BBR substantially reduced the expression of proinflammatory cytokines, namely, IL-6, MCP-1, TNF-α, and IL-1β. Furthermore, BBR could inhibit the expression of ER stress genes, including ATF4, CHOP, and XBP-1.

C-C motif ligand 19 (CCL19), known as macrophage inflammatory protein-3β (MIP-3β) as well, is expressed by dendritic cells and macrophages and contributes to chronic inflammation [8, 29, 54]. Studies have indicated that BBR promotes the expression of phosphorylated-AMPK (p-AMPK) by suppressing CCL19, which in turn inhibits SREBP-1c [8, 23, 29, 48, 54]. Another possible anti-inflammatory effect of BBR in NAFLD is by inhibiting the NLRP3 inflammasome signaling pathway [28]. Inflammasomes, as a group of multimeric protein complexes, promote the expression of proinflammatory cytokines namely Il-18 and IL-1β by activating caspase-1 during infection, metabolic imbalance, and tissue damage [83]. Several studies have confirmed the effects of NLRP3, as an important inflammasome, on liver injury and hepatic fibrosis. It has been demonstrated that BBR administration could suppress the activation of inflammasomes by affecting the purinergic (P2X7) receptor [28]. It has been demonstrated that BBR may ameliorate nonalcoholic steatohepatitis and hepatoprotective effects by restoring the Treg/Th17 ratio, reducing lipid accumulation, and regulating the chemerin/CMKLR1 signaling pathway to attenuate hepatic inflammation. Oxidative stress is resulting from an imbalance between the cell's redox environment and antioxidant defenses in human tissues [1, 84]. In NAFLD, the elevated levels of free FFA in the liver promote the production of ROS, mitochondrial dysfunction, and ATP depletion, which leads to oxidative stress [14, 52]. Several possible mechanisms have been proposed for the antioxidant effect of BBR. Antioxidant effects of BBR could be through Sirt 3 activation [36]. Sirt 3 contributes to energy metabolism by regulating the acetylation of mitochondrial enzymes [37]. BBR improves mitochondrial function, particularly at least, through Sirt 3 activation, which consequently reduces the ROS generation [36, 37]. Furthermore, it has been demonstrated that BBR inhibits the over-production of ROS by suppressing the expression of Nox-2 [85]. The NADPH oxidase-2 (Nox-2), one of the NADPH oxidase family members, contributes to the generation of cytoplasmic ROS by generating superoxide in NAFLD patients, which result in hepatic steatosis [85]. In a study by Sun et al. [14], 10 μM of BBR in Huh7 and HepG2 could substantially mitigate the expression of Nox2, Nrf-2, HO-1, and SOD. Of note, the findings of another study by Rafiei et al. [30] demonstrated that BBR (10 μM) had no impact on the expression of iNOS in HepG2.

Of note, BBR inhibits the mitochondrial ROS generation by downregulation of complex I and II in the electron chain. On the other hand, BBR upregulates complex V expression, which indicates that it does not attenuate ATP generation [14].

3.4. Strengths, Limitations, Future Directions, and Knowledge Gaps. The present study has both limitations and strengths. To the best of our knowledge, this is the first systematic review investigating the effects of BBR supplementation and mechanism of action on patients with NAFLD. BBR and metformin have similar effects in controlling hemoglobin a1c levels. They also have similar effects on fasting blood glucose levels and postprandial blood glucose levels [86]. Glucosyl is a BBR supplement. When glucosyl and metformin are taken together, it has an additive effect [87]. This means that hemoglobin a1c, fasting blood glucose, and postprandial glucose levels decrease further. Metformin lowers hemoglobin a1c, but it also lowers body weight. When it comes to BBR and metformin for weight loss, metformin appears to be superior to BBR, also helping to reduce the risk of death from cardiovascular disease [88]. Both metformin and BBR support healthy blood sugar levels. They are similar in terms of their mechanism of action and their effect on the body at the cellular level. Metformin and BBR both increase AMPK enzyme activity [87]. The study reviewed both animal and human studies with sufficient sample sizes. Moreover, the duration of interventions was between 1 hour and 16 weeks, and different doses of BBR supplementation were administered. In spite of its strengths, the main limitation of the study was the heterogeneity of selected studies, which could be due to the variation in doses, study durations, etc. Another notable limitation was entering English publications only. Although several *in vitro*, *in vivo*, and animal studies, particularly rat and mouse models, have shown the beneficial effects of BBR on NAFLD, more clinical trials and animal experiments are required, before conducting large-scale endpoint studies. Of note, HFD-induced NAFLD animal models can simulate the mechanisms and pathogenesis of NAFLD in humans, yet there might be different histopathology, which should be confirmed. Due to the administration of various dosages of BBR in experimental studies, the lack of information regarding the required dosage ranges is considerable, which is needed to be elucidated. Moreover, there is a substantial lack of information on the possible side effects of BBR supplementation in both animal and human tissues. For example, Sun 2017, et al. demonstrated that BBR supplementation (200 mg/kg/d in rats and 300 mg/kg/d in mice for 8 weeks) could suppress the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) in hepatic cells. The Nrf-2 is a transcription factor, which is activated in the presence of ROS and promotes the expression of antioxidant enzymes including HO-1 by translocation to the nucleus. As a result, in

a bid to identify probable side effects of BBR, further experimental studies are essential. It should be mentioned that due to the heterogeneity of the studies and particularly few available human studies, the current review could not be a meta-analysis study.

3.5. Side Effects. Despite the beneficial effects of BBR against various diseases, in particular NAFLD, some studies have reported the incidence of side effects regarding the administration of BBR at higher doses [38]. It seems that BBR at low doses is safe for both the animal and human body. Also, taking high doses of BBR may cause digestive disorders such as diarrhea, constipation, abdominal pain, and bloating [89, 90]. Evidence has suggested that BBR is well tolerated at the dosage of 0.3 g/daily in combination therapy [91].

4. Conclusion

The present systematic review exerted the beneficial effects of BBR on NAFLD and its associated metabolic disorders including dyslipidemia, obesity, and insulin resistance. The results of the study have demonstrated the noteworthy antihyperlipidemic effects of BBR, which are attributed to its ability to regulate lipid metabolism, facilitate the β-oxidation of fatty acids, and mitigate the accumulation of triglycerides in hepatocytes. In addition, it has been observed that BBR has a positive impact on NAFLD through the regulation of inflammatory signaling pathways, inhibition of oxidative stress, and reduction in the excessive production of cytoplasmic and mitochondrial ROS. The therapeutic properties of BBR in NAFLD and its associated complications have been observed in various studies, indicating its potential as a potent medication for NAFLD management.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

AK, IN, VA, SS, PSMA, and FD developed the first hypothesis of the study and searched the data, and both authors assessed and extracted data. AK, PSMA, and MV wrote the draft of the manuscript. AK and PSMA contributed to data collection; MV provided advice and consultation; AK contributed to the final revision of the manuscript.

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