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I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

Mohamed L. Salem,

Editor in Chief

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

REVIEW ARTICLE

Metformin: An AMPK-dependent antidiabetic drug with novel medical applications

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ABSTRACT

Metformin (MET) is a well-known antidiabetic drug for type 2 diabetes mellitus (T2DM) treatment. The therapeutic effects of MET come from its ability to decrease both the production of hepatic glucose and its absorption via the intestine and improve insulin hormone sensitivity. This review aims to summarize the new medical applications of this old antidiabetic drug. Nowadays, MET is used in pre-clinical trials to treat cancer and slow the proliferation rate, emerging from its hypoglycemic effect and antioxidant activity. MET enhanced the functions of immune T-cells and reduced the proinflammatory mediators in macrophages. Moreover, based on the pre-clinical studies, its effect on the reproductive system is confirmed as MET showed an excellent candidate for polycystic ovary syndrome (PCOS) treatment and improved ovarian cyclicity, and reduces gestational diabetes risks in female; however, the effect on sperm number and motility is still in debate**.** Treatment with MET also showed some beneficial effects on bone health, and its administration led to a deficiency in vitamin B_{12}. Furthermore, MET enhances anaerobic pathways and increases lactic acid levels. This review demonstrated the novel uses of MET in different clinical settings with explained mechanisms of action.

Keywords: Anticancer; antioxidant; anti-diabetic; obesity; Metformin.

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INTRODUCTION

Metformin (MET) is a well-known anti-hyperglycemic agent for type 2 diabetes mellitus (T2DM) management, which was originated naturally from galegine found in *Galega officinalis* (Bailey and Day, 2004). MET has been used as glucose-lowering agents via reducing gluconeogenesis in hepatocytes, decreasing glucose absorption from the intestine, and restoring the body's response to insulin in peripheral tissues (Viollet et al., 2012). MET is a unique antidiabetic drug not only because it does not cause severe hypoglycemia but also because it can improve protein synthesis. MET was discovered in 1922 (Werner and Bell, 1922). Between 1940-1957s, it was used in malaria, influenza, and adult-onset diabetes treatments. From 1995-1998s, MET was applied in clinical uses in the USA and UK to manage hyperglycemia in T2DM. In addition to its use as an antidiabetic drug, MET was recently used for another clinical setting, such as obesity treatment, anticancer, antioxidant, hepato-, and reno-protective (Scheen et al., 2015; Chukwunonso et al., 2016). Besides the previous MET uses, it can also be used simultaneously as a cardio-protective agent, augment the immune system, and increase probiotics' efficiency (Nasri, 2013).

Mechanisms of Action of Metformin

MET is usually taken orally with a wide therapeutic dose range from 1,000 to 3,000 mg/day. About 70% of MET is absorbed approximately from the gastrointestinal tract to the body tissues through the bloodstream, the remaining passing into the colon then excreted in feces (Graham et al., 2011). The absorption of MET is mediated primarily by the plasma membrane monoamine transporter in the intestine. However, the organic cation transporters 1 and 3 may also contribute to the absorption process (Gong et al., 2012).

Previous studies showed that MET inhibits mitochondrial complex I, oxidative phosphorylation, ATP production, and energetic stress (Gheissari, 2013; Rena et al., 2017). In this regard, the metabolic shift by MET leads to the activation of 5'-adenosine monophosphate-activated protein kinase (AMPK), subsequently retrieves energy balance by switching on ATP-producing catabolic pathways and switching off ATP-consuming anabolic mechanisms (Rena et al., 2017). Furthermore, MET enhances anaerobic pathways by inhibiting pyruvate dehydrogenase (PDH), glycerophosphate dehydrogenase, and mitochondrial reducing agents. Inhibiting glycerophosphate dehydrogenase by MET leads to a decrease in both lactate and glycerol metabolism, which in turn decreases glucose biosynthesis and increases lactic acid levels (Zoncu et al., 2011).

The mammalian target of rapamycin (mTOR) is a downstream target of AMPK involved in regulating energy homeostasis by modulating cellular processes, such as protein synthesis and autophagy implicated in cell proliferation and tumorigenesis in cancer (Madiraju et al., 2014). MET has been demonstrated to downregulate mTOR signaling through either AMPK-dependent or independent mechanisms (Kalender et al., 2011). As shown in Figure 1, MET can regulate other pathways, including the nuclear factor kappa β (NF-kβ) (Chaudhary et al., 2012) and mitogen-activated protein kinase (MAPK)/c-Jun NH_2-terminal kinase (JNK). Further, MET blocked the adenylyl cyclase enzyme, which in turn inhibited glucagon-induced cAMP production.

Metformin and Oxidative Stress

MET showed antioxidant activity, suggesting its potentiality against several diseases (Esteghamati et al., 2013). Interestingly, decreasing insulin sensitivity is linked with low mitochondrial protein abundance and associated with reactive oxygen species (ROS) accumulation. Several studies have indicated that MET treatment led to decreased ROS cellularity, which protects against tissue injury (Rouhi and Ganji, 2013). The action of MET in reducing ROS generation might be due to the suppression of pro-oxidant enzyme systems like NADPH oxidase that cause superoxide anions production (Piwkowska et al., 2010). As a result of hyperglycemia in diabetic patients, a progressive accumulation of cellular ROS accompanied by advanced glycation end products (AGEs) was significantly noticed. MET treatment has been found to suppress AGEs by enhancing the antioxidant capacity (Chakraborty et al., 2011). A previous study indicated that MET ameliorated endoplasmic reticulum (ER) stress-induced by albumin in human proximal tubular cells by ROS-Src-kinase-mTOR pathway through suppression of ROS generation and augmentation of antioxidants status (Azimova et al., 2014). The antioxidant effects of MET could mediate through AMPK activation, which led to reducing ROS and inhibiting profibrotic/proinflammatory factors. In the liver tissue, a weak antioxidant defense system and oxidative stress led to the hepatic lesion. A recent study showed that co-treatment with MET and propolis attenuates hepatic lesions in DM rats (Nna et al., 2018).

Metformin, Immunity, and Inflammation

Due to the immune-modulatory features of MET, it may be used in future trials in the treatment of immune-mediated inflammatory diseases (Pollak, 2017; Schuiveling et al., 2018). MET led to the activation of AMPK downstream. Therefore, it influenced immune functions and homeostasis. Studies suggested that MET treatment may affect different acquired and innate immune cellularity (Schuiveling et al., 2018). MET exerts several actions on T helper 17/regulatory T cell (T-reg) balance, cytokine synthesis, macrophage polarization, autoantibodies production, neutrophils release, and extracellular matrix remodeling (Tomczynska et al., 2016). T cells' differentiation and activation depending on the mTOR signaling via activation of the STAT transcription pathway (Saleiro and Platanias, 2015). In this regard, MET treatment can reduce adenovirus-induced acute liver injury partially mediated through the mTORC1 pathway in T lymphocytes in mice. It has also been reported that MET increases the number of T-reg cells in experimental autoimmune encephalomyelitis (EAE) through the inhibition of the mTOR pathway (Sun et al., 2016).

Similarly, the effect of MET on collagen antibody-induced rheumatoid arthritis has been addressed (Kang et al., 2013). Further studies have been evaluated the impact of MET on Th17-mediated inflammation, acute graft-versus-host, and colitis disease (Lee et al., 2015; Park et al., 2016). MET co-treatment with drospirenone/ethinylestradiol in polycystic ovary syndrome (PCOS) patients has been shown to decrease $CD4^{+}CD28^{null}$ T cells frequency. It was reported that the MET antitumor action was mediated by AMPK activation in lymphoma (Shi et al., 2012). Furthermore, MET administration has been accompanied by an improvement in the response rate in diabetic diffuse large B-cell lymphoma patients and attenuate autoimmunity signs (Lee et al., 2017).

In vitro study showed that MET had anti-inflammatory properties and inhibited LPS-induced pro-IL-1β in murine bone marrow-derived macrophages (Jing et al., 2018). Moreover, MET treatment has been shown to inhibit collagen synthesis and fibrosis *via* the TGF-β/Smad3 signaling pathway in cardiac fibroblasts. A similar effect was observed in other cellular models, hepatic stellate cells and renal fibroblasts (Lu et al., 2015). Similarly, in the lungs, MET attenuates pulmonary fibrosis and reduced collagen accumulation. MET administration is linked with a decrease in the production of TGF-β, a well-established inducer of fibrosis (Li et al., 2015).

Metformin and Obesity

MET has been used to control overweight in diabetic patients (Wang et al., 2017). MET treatment interestingly showed decreased low-density lipoproteins (LDL-bad cholesterol) and triglycerides levels, which promotes weight loss in obese patients. It activates lipolysis by inhibiting adipogenesis and uncoupling proteins (Després, 2003). Indirectly, MET inhibits carbohydrate absorption and bile salt uptake through stimulation of glucagon-like peptide-1 (GLP-1), which may inhibit food intake and energy production (Petersen et al., 2017). Treatment with MET is linked with an increase in fatty adiponectin that enhances fatty acid oxidation in T2DM patients (Nie and Li, 2017). In parallel, MET inhibits rapamycin's mammalian target (mTOR), which in turn mitigates insulin's pro-obesogenic activity (Kalender et al., 2011).

Hepatoprotective Effect of Metformin

MET might protect the liver against viral and chemical hepatotoxicants via different mechanisms, including activation of AMPK and inhibition of mitochondrial NADH dehydrogenase complex I, MAPK, and Smads phosphorylation (Iranshahya et al., 2019). MET shifted the metabolic status in hepatocytes toward the inhibition of glucose, lipid, protein synthesis, and activation of fatty acids oxidation and glucose uptake (Rena et al., 2017). Recent studies reported that MET might also protect the liver cells from injury induced by toxic substances such as thioacetamide, alcohol, methotrexate, acetaminophen, cisplatin, and arsenic trioxide (Borole et al., 2016; Saeedi Saravi et al., 2016; Mansour et al., 2017; Al-Hashem et al., 2018). Since the liver is the main organ to eliminate acetaminophen (Mobasher and Valverde, 2014), MET successfully protected hepatocytes from acetaminophen toxicity via MAPK, non-MAPK, and inhibited JNK activation (Kim et al., 2015). MET prevented liver fibrosis and inflammation in CCl4 induced cirrhotic rats (Xu et al., 2016). Using Continuous treatment with MET in cirrhotic T2DM patients increased their survival rate. MET could have a beneficial impact in non-alcoholic fatty liver patients regardless of its effects as an insulin sensitizer (Mazza et al., 2011). The hepatoprotective effect of MET might be due to the enhancement of antioxidant responses by increasing glutathione reductase (GR), glutathione peroxidase (GPx), and cysteine synthase levels and upregulation of nuclear factor (Nrf2) and suppression of transforming growth factor-beta 1(TGF-β1) (Iranshahy et al., 2019).

Metformin and Diabetes Mellitus Fighter

MET is commonly used to treat T2DM patients and prediabetic patients (Ortega et al., 2014). Comparing to insulin and chlorpropamide therapy, MET showed advancement in controlling diabetes complications and mortality. Upon MET treatment, the Incidence of T2DM decreased by 34% (Nasri et al., 2013). Several mechanisms showed the ability of MET to reduce serum glucose levels in T2DM

patients (Konopka et al., 2019). For instance, it improves insulin sensitivity via increasing peripheral glucose uptake and utilization. Besides, it also decreases hepatocytes' capability to produce glucose by reducing the rate of gluconeogenesis (Wiernsperger and Bailey, 1999) and glycogenolysis without induction of hypoglycemia. Although the molecular mechanism of metformin action has not been well described, some studies triggering activation of AMPK as the primary mechanism of protection (Shaw et al., 2005). However, other researchers have shown that AMPK is not involved. Furthermore, in muscle tissues, MET stimulates glucose transport and insulin signaling (Seo-Mayer et al., 2011). Clinical studies supported the capability of MET to reduce the incidence of myocardial infarction, angina, stroke, peripheral vascular disease, and sudden death in T2DM patients (Azimova et al., 2014; Kooy et al., 2009; Lamanna et al., 2011). Besides the previous actions of MET, it increases the peripheral glucose disposal that arises by increasing non-oxidative glucose disposal into skeletal muscle (Nasri et al., 2013).

The therapeutic and renoprotective efficacy of MET against nephrotoxicity was previously investigated by Rafieian-Kopaei and Nasri (2013), Baradaran (2012), and Eisenreich and Leppert (2017) (Rafieian-Kopaei and Nasri, 2013; Baradaran, 2012; Eisenreich and Leppert, 2017). Several studies have reported that MET could inhibit hyperglycemia on AMP-activate protein kinase (AMPK) signaling in kidney glomerular and tubular compartments. Furthermore, MET was able to ameliorate the renal injury induced by hyperglycemia (Takiyama et al., 2011; Lee et al., 2013). It has been reported that MET significantly decreases albuminuria in DM patients and protects against the podocytes, which could be a promising agent in diabetic nephropathy management (Nasri and Rafieian-Kopaei, 2013; Tavafi, 2013). Furthermore, MET protects against gentamicin-induced acute renal failure and after unilateral ischemia-reperfusion in rats (Amini et al., 2012). Taheri et al. (2012) reported that activation MET could prevent hypoxia and protects renal injury via inhibition of mitochondrial respiratory complex I (Taheri et al., 2012). MET has been shown to induce autophagy by phosphorylating key proteins ULK1, VPS34, and Beclin 1. These findings could encourage physicians to use MET in diabetic nephropathy control. Another study showed that MET might cause side effects on renal function in T2DM patients and moderate chronic kidney diseases due to lactate accumulation, which led to lactic acidosis (LA) (Hsu et al., 2017). Recently, a new study reported that MET could be applied safely in T2DM patients with chronic kidney diseases after optimizing its dose (MacCallum and Senior, 2019).

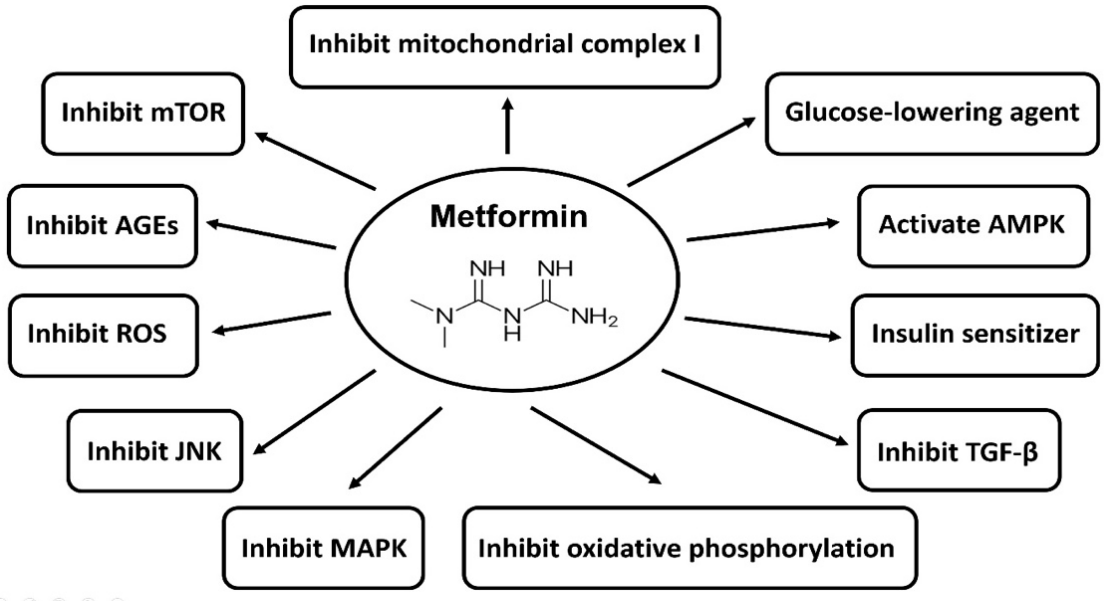

Figure 1. Metformin inhibitory and stimulatory effects.

Metformin and Cardiovascular Health

Pre-clinical and clinical studies showed that MET has a potential role in lowering cardiovascular complications in diabetic and non-diabetic patients (Luo et al., 2019). Diabetic patients are most likely subjected to risk factors of cardiovascular diseases (CVD) development due to the changes in the internal environment, which directly or indirectly affected heart functions. Controlling the hyperglycemia in diabetic patients is ultimate in CVD management (Avogaro et al., 2007). It has been reported that MET was also able to reduce atherosclerotic cardiovascular disease in T2DM patients (Charytanet et al., 2019). In the same line, previous studies suggest that MET exhibited antifibrotic effects with the potential impacts on cardiovascular disease (Lu et al., 2015; Shen et al., 2016). MET can reduce the myocardial infarction (MI) incidence in freshly diagnosed obese T2DM patients (Varjabedian et al., 2018).

Similarly, MET can potentially limit ischemia-reperfusion injury in MI animal models. Several studies have recently postulated that MET positively impacts a cardiovascular protecting agent (Younis et al., 2017; Charytan et al., 2019) because MET showed to reduce blood fats, body weight, and blood pressure (Van Stee et al., 2018). In a contradictory study, MET could be unsafe to use as a treatment for diabetic patients with cardiac shortage because it may increase the risk of LA (Inzucchi et al., 2014).

Metformin and Reproductive Health

The menstrual cycles and fertility might be improved by MET treatment. MET showed an excellent candidate for PCOS treatment and improved ovarian cyclicity, and reduces gestational diabetes risks (Brown et al., 2016; Vanlalhruaii et al., 2018). MET increased the pregnancy rates and decreased the ovarian hyperstimulation syndrome risk (Tso et al., 2015). MET reported regulating oocyte maturation. It is considered a promising contributor in the crosstalk between somatic cells and oocytes for the normal development of female germ cells and embryos. MET influences the cell cycle by altering the Follicle-stimulating hormone-induced proliferation and cyclin-dependent kinase inhibitor in the primary culture of mouse Sertoli cells (Riera et al., 2012). In obese patients, MET treatment improves sperm concentration and motility in the same way observed in obese rats (Morgante et al., 2011). MET can modulate and improve pituitary Luteinizing hormone (LH) and regulate Leydig cell steroidogenesis in testis. MET decreases sperm motility in pigs (Hurtado et al., 2012). In the rabbit, the MET treatment showed a negative effect on concentration, mobility, and the number of morphological abnormalities of spermatozoa (Naglaa et al., 2010). In birds, MET administration improved their sperm viability and mobility (Nguyen et al., 2014).

Metformin and Cancer

Cancer cells are characterized by their rapid proliferation; therefore, they use a huge amount of glucose (Jang et al., 2013). Depletion of glucose or reducing its amount from cancer cells is an excellent strategy to slow the cancer cells' growth or inhibit their proliferation (Ryu et al., 2014). Several researchers around the world use MET as a promising candidate for cancer treatment (He et al., 2015; Saraei et al., 2019), and our research group is getting similar results in hepatocellular carcinoma (HCC) with pre-existed diabetes mellitus rats (Mobasher et al., 2020; Mobasher *et al .,* 2021). Multiple scientific reports linked the presence of diabetes mellitus and cancer incidence (Wang et al., 2015). Hyperglycemia is a key factor for rapid cell proliferation and directly impacts carcinomas' cell proliferation, apoptosis, and metastasis (Ryu et al., 2014). In this regard, diabetic patients are more susceptible to cancer than non-diabetics, and MET may help prevent tumor formation. In this line, reports have concluded that the treatment of diabetic patients with MET could decrease the risk of cancer development and progression as compared to those using other antidiabetic drugs (Libby et al., 2009). For instance, MET treatment protects against pancreatic cancer. MET inhibits renal cancer cell proliferation by down-regulating cyclin-D1, inducing G0/G1 cell cycle arrest, and upregulates AMPK activity along with mTOR pathway inhibition (Libby et al., 2009). MET also has destructive effects on osteosarcoma cancer stem-like cells, and potentially, it can reduce the risk of cancer in continuous use by T2DM patients (Chen et al.,

2015). An epidemiological study suggested that MET inhibits mTOR-dependent translation initiation in breast cancer cells (Dowling et al., 2015). Simultaneously, other studies showed that MET has potential antitumor activity against lung, breast, and colon, pancreatic, prostate, and ovarian cancers (Zheng et al., 2018; Zaidi et al., 2019).

Metformin enhances probiotics

Probiotics are living microorganisms that play an essential role in health and disease. They improve the immune system and decrease blood glucose via improving inflammation and preventing β-cells destruction. Furthermore, probiotics may have beneficial effects on glycemic control and lipid profiles in T2DM patients (Bayat et al., 2016). MET showed an impact on some microorganisms of human gut microbiota by influencing folate production and, consequently, inhibiting the growth of some types of bacteria (Olgun, 2017). People with diabetes administrated with MET showed alterations in their microbiota with an abundance of lactobacilli and bifidobacteria, and these species could contribute to the antidiabetic effect (Wu et al., 2017). A previous clinical trial found that combinatorial treatment with probiotics and MET improves liver function better than MET alone in non-alcoholic steatohepatitis patients (Shavakhi et al., 2013). Several studies on rodents and humans suggest that gut microbial changes might contribute to the antidiabetic effect of MET (Forslund et al., 2015; De la Cuesta-Zuluaga et al., 2017).

Metformin and Bone Health

MET does not affect glucose levels in non-diabetic individuals, and this represents the idea of using MET as adjuvant therapy, especially in bone disorders. MET has a beneficial impact on bone health by controlling glucose levels that result in enhanced bone turnover, which leads to an imbalance of osteoblast/osteoclast activity (Stage et al., 2018). Patients with T2DM have reported lower biochemical bone turnover markers (Manavalan et al., 2012). MET has been shown to shift the progenitor cells into osteoblasts and showed direct osteogenic effects on bone through AMPK and Runt-related transcription factor 2 (Runx2) and indirect effects by hyperglycemic correction (Molinuevo et al., 2010). A previous study has reported that AMPK promotes MC3T3-E1 cells for osteogenesis via the AMPKGfi1- OPN axis pathway (Wang et al., 2016). Another research has suggested that AMPK can suppress RANKL-induced osteoclast formation (Lee et al., 2010). The previous study has shown that MET can be osteogenic *in vitro* following by activation of AMPK resulting in osteoblastic cells' differentiation, bone matrix synthesis, and osteoblasts proliferation (Shah et al., 2010). *In vitro* and *in vivo* studies showed that MET administration has osteogenic effects and improves bone healing in non-diabetic animals. In diabetic and non-diabetic rats, MET led to an increase in type I collagen synthesis and stimulates the regeneration process in bone lesions (Molinuevo et al., 2010).

Furthermore, MET osteogenic effects were presumably mediated by the induction of nitric oxide synthases (NOS) and extracellular signal-regulated kinase activation (ERK). MET has protective effects against bone loss after ovariectomy in rats and protects bone mass in estrogen deficiency. It has also been reported that MET can increase bone density and mineralization in alveolar bones through osteoblast differentiation in ligature-induced periodontitis in rats (Bak et al., 2010).

Metformin toxicity

The common symptoms of MET toxicity include nausea, abdominal pain, vomiting, hypothermia, decreased level of consciousness. One of the most serious side effects of MET treatment is the development of LA due to its blockage of the metabolism of lactate and alanine to pyruvate, which in turn led to the collapse of the cardiovascular system (Wang and Hoyte, 2019). Treatment of MET toxicity is symptomatic and supportive, and there is no antidote available, but LA is commonly treated by sodium bicarbonate or through hemodialysis (Turkcuer et al., 2010). Furthermore, MET impaired vitamin B_{12} uptake in the terminal ileum (Bauman et al., 2000) and folic acid. MET treatment in some cases led to hemolytic anemia, which could be potentiated by immune response or due to glucose-6-phosphate dehydrogenase (G6PD) deficiency. Besides its metabolic effect, MET treatment is linked with

a reduction in proteins of coagulation and impaired fibrinolytic activity in T2DM (Ruggiero et al., 2016). Furthermore, MET intoxication led to alterations in specific coagulation parameters. Serious side effects of this drug, such as idiosyncratic hepatotoxicity, were also reported (Miralles-Linares et al., 2012).

CONCLUSION

MET has been widely prescribed to T2DM patients for over 50 years and considered a safe and effective drug when used alone and in combination with other oral antidiabetic agents, with several benefits such as decreased hyperinsulinemia, weight reduction, and improve lipid profile. Recent results also indicated the nephroprotective activity of MET against nephrotoxic agents and demonstrated its effectiveness in reducing the complications associated with nephropathy in chronic kidney diseases. Recently, MET might be used to treat several types of cancer. Different studies indicated that MET is not exclusively used as an antidiabetic drug but also has antioxidant effects, the ability to decrease the complications associated with cardiovascular diseases in diabetics, augment the immune system and probiotics in the gastrointestinal tract.

CONFLICT OF INTEREST

All authors have approved this article and declare no conflicts of interest.

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