# A Review on Potential Anticancer Properties of Antidiabetic Drugs: Metformin and Sulfonylureas

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## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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# Approval

The thesis titled "A Review on Potential Anticancer Properties of Antidiabetic Drugs: Metformin and Sulfonylureas" submitted by Tamara Hossain (19346070) of Summer, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on March, 2024.

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## Ethics Statement

This study does not involve any human or animal trial.

## Abstract

Diabetes mellitus is a public health emergency that is increasing drastically worldwide. In many research findings, it is shown that it causes not only cardiovascular diseases but also increases the risk of cancer. Metformin and sulfonylureas are commonly used drugs in the treatment of type 2 diabetes and recent meta-analysis also emphasizes their effects against various types of cancer like breast cancer, prostate cancer and pancreatic cancer. Metformin activates the AMPK and mTOR pathway and it also blocks the mitochondrial respiratory chain complex. Consequently, metformin inhibit the protein synthesis and cell proliferation of tumor cells. The second generation of sulfonylureas are more potent and also exhibit anticancer effects. Sulfonylureas block K+ ion channels, so depolarization occurs thus activating caspase 12 which activates caspase 3,6,7 thus resulting in tumor cell apoptosis. This review article is significant and is expected to realize a much needed knowledge gap in examining the antineoplastic effects of metformin and sulfonyl ureas for drug repurposing.

**Keywords:** Merformin, Sulfonylureas, Anticancer drugs, Mitochondrial respiratory, Caspase, Drug repurposing.

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## List of Acronyms

AMPK: AMP-activated protein kinase

mTORC1: Mammalian target of rapamycin complex 1

PKB: Protein kinase B

Rheb: Ras homologue enriched in brain

TSC2: Tuberous sclerosis complex 2

LKB1: Liver kinase B1

PTEN: Phosphatase and tensin homologue deleted on chromosome 10

ABC: Adenosine triphosphate transporter

Chemo: Chemotherapy

IGFR: Insulin growth factor receptor

Akt: Protein kinase B

IGF: Insulin growth factor

IR: Insulin receptor

Pl3K: Phosphoinositide 3-kinase

IKK: I kappaB kinase

MMP: Matrix metalloproteinase

TNF: Tumor necrosis factor

VEGF: Vascular endothelial growth factor

TNFR: Tumor necrosis factor receptor

ATP: ATP sensitive potassium channel

LPS: Lipopolysaccharide

# Chapter 1

# Introduction

## 1.1 Diabetes and metformin

Diabetes is a chronic, health condition that affects the body. The body breaks down food and produces sugar which goes into the bloodstream. When blood sugar rises up then it signals the pancreatic receptor to release insulin and insulin reduces the sugar level from blood. Unfortunately, people with diabetes their pancreas does not release enough insulin and also does not produce enough insulin which is to be released. When sugar levels rise into the bloodstream their insulin receptor does not respond which can cause serious health issues like kidney failure and heart failure (Amin et al., 2019; Zhou et al., 2018). The full cure for this condition has not been discovered yet. However, healthy food, exercise, and weight loss can control diabetes. Data shows around 38 million adults have diabetes where 1 in 5 does not know about it. In the USA the eighth leading cause of death is diabetes. Additionally, it is the number one cause of kidney failure. Diabetes in adults has doubled in the last 20 years (Vancura et al., 2018). There are three types of diabetes: type 1, type 2 and gestational diabetes. Type 1 diabetes occurs due to autoimmune reactions where one's own body system attacks its own body function mistakenly. Additionally, it stops producing insulin due to this reaction. Around 5 to 10 percent of people have this type 1 diabetes. At any age it could be diagnosed. No one can live without insulin in type 1 diabetes. Nowadays, how to cure type 1 diabetes can not be known. On the other hand, in type 2 diabetes the body does not properly function on insulin. More than 90 to 95 percent of diabetes patients have type 2 diabetes. People do not know the symptoms at a first phase but people need to control the sugar level in blood for avoiding future health complications. Type 2 diabetes can be controlled by changing the lifestyle such as exercise, healthy food, eating habits, weight loss. In pregnant

women gestational diabetes can be seen. If a mother has gestational diabetes it might be spread to her babies. Moreover it enhances the risk of type 2 diabetes after birth. In the USA around 98 million people more than 1 in 3 people might have prediabetes. In prediabetes the sugar level is high in blood but people have not known about their consequences (Chae et al., 2016; Nayan, et al., 2015).

Cancer is an uncontrolled cell growth in any part of the body and spread all over the body through the bloodstream. Basically, human cells grow and old cells are damaged then those new cells take over. Sometimes, cell division is hampered when cells grow rapidly in any parts of the body and form tumors. Moreover, this tumor sometimes can be cancerous or not cancerous. Cancerous cells then spread to other parts of the body through the bloodstream and damage the healthy cells. Tumors are two types: benign tumor and malignant tumor where benign tumor does not spread and also does not grow rapidly. Also it does not cause cancer. On the other hand, malignant tumors grow rapidly and spread to other parts of the body. Cancer is caused by genetic material disbalance that affects the cell function. The DNA damage of normal cells is due to some harmful substance like environmental pollution, chemical substance, radiation, ultra violet rays etc. Cancer affects the three main types of genes like tumor suppressor genes, proto oncogenes, DNA repairing genes. Tumor suppressor genes work to control cell division but cancer altered the genes so the cells grow uncontrollably. Proto oncogenes work to control cell growth but cancer can alter those genes so the cells grow rapidly. DNA repairing genes can repair the damaged gene. But cancer can affect the repair genes and it may cause mutation. Genetic mutation can cause cancer. Cancer spreads when they undergo a process called metastasis. Cancer is one of the second leading causes of death. In 2017 almost 46 percent of cancer patients died at the age of 70 or more. 41 percent of cancer patients died between 50 to 69 years old. In 2023 almost 609,820 people died due to cancer in the USA. Curcumin has been used as a traditional anticancer agent

throughout thousands of years. They are extracted from roots of the curcuma longa plants. Many preclinical trials have shown that it has anticancer properties and it is also safe.

Metformin is a drug which is used to treat diabetes mellitus that is approved by the FDA. Metformin is differ from other antidiabetic drugs like sulfonylureas. Metformin doesn't work to increase the insulin secretion and not produce hypoglycemia. Metformin decreases the glucose level in blood by increasing the skeletal muscle uptake of glucose and also decreasing the absorption of glucose. Additionally, maximum dosage of drug gives the synergistically control of the glucose level. The drug has other benefits like weight loss. For patients with peripheral vascular disease it improved the blood flow and it also improved the lipid profiles. It is normally safe if it is administered according to the prescriber. Recent studies have shown the beneficial effects of metformin as potential anticancer drugs. Metformin suppresses the tumor cells and it enhances the activity of T cells. It also damages the gene mutation. Many studies show that metformin has anticancer agent when it is used with other anticancer treatment. Metformin also works on hyperinsulinemia which is associated with cancer. Diabetes and obesity which are related to cancer such as enhancing the growth factor, insulin resistance, hormonal disbalance etc. The antidiabetic drugs are commonly used world wide for diabetic patients and it has vast therapeutic effects such as in anticancer effects. So the antidiabetic drug, metformin prevents the risk factor of cancer and cures cancer (Nasri & Kopaei, 2014). This review article looks into the scientific evidences in support of metformin and sulfonyl ureas, another class of antidiabetic drugs as potent antineoplastic drugs besides their antihyperglycemic effects.

## 1.2 Objectives

The main objective of this study involving the potential anticancer effect of two antidiabetic agents metformin and sulfonylureas is fill up much needed knowledge gap in the literature of

potentially repurposing antihyperglycemic drugs towards treating cancer. Researchers are very curious about the topic and they explored the impact of these two classes of antidiabetic drugs in various types of cancer but a thorough literature survey is lacking that can aid others to get introduced into this area. This review article found that both class of antidiabetic agents has great influence on inhibiting cellular growth and cell proliferation, reducing cellular metabolism and inactivating inflammation pathways.

Among the studies performed regarding the topic, some of the study results shows significant impact of these drugs in the treatment of cancer. However, more studies and more clinical trial is required in order to find the optimal information of these two drugs and whether they are safe for use in humans or not as revealed by the review article. Researchers are working on depth to understand the precise mechanism of these drugs along with potential clinical application in cancer treatment. Furthermore, by examining their impact on cancer cells, researchers aim to uncover any therapeutic benefits these medications may offer in the context of cancer treatment. Understanding how these drugs interact with cancer cells could provide valuable insights into novel treatment approaches or combination therapies for cancer patients, potentially leading to improved outcomes and quality of life and this review article also summarizes some of these studies as well.

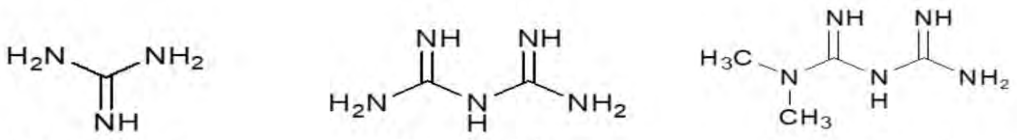
## 1.3. Methodology

The review work is summarized from various relevant literatures. The information and data for this review came from various articles and academic journals which were found on the digital libraries. Examples of some resources are Frontiers, PubMed, Google Scholar, Science Direct, Research Gate, MDPI and other various relevant databases. The relevant topic from the articles were found and the further important topics were looked into. After analyzing the

data outline was created with all necessary information. PubMed is a free online research tool that works like a digital library from which helps me to get many valuable information like the history of metformin and sulfonylurea, mechanism of action. All the clinical study data that is present in my writing is collected from PubMed. All the general structure of metformin and sulfonylureas were collected from national library of medicines. Google Scholar is a very necessary tool to find a large database of scholarly articles which helps to find relevant information on the topic. The Science Direct provides me with the latest information about the topic which helps to enhance my knowledge in the medicinal field. The online journal MDPI is a user-friendly research tool that provides relevant data on the topic. Manually APA in-text citations were performed on the essay.

## 1.4 Metformin and its Derivatives

**Structure:** Two guanidines are attached together and form biguanide. In metformin structure two methyl groups are added to the nitrogen. Metformin reduces glucose absorption from the GI tract and also increases the uptake of glucose in the muscle. That is how it is more antihyperglycemic than hypoglycemic (Graham et al., 2011).



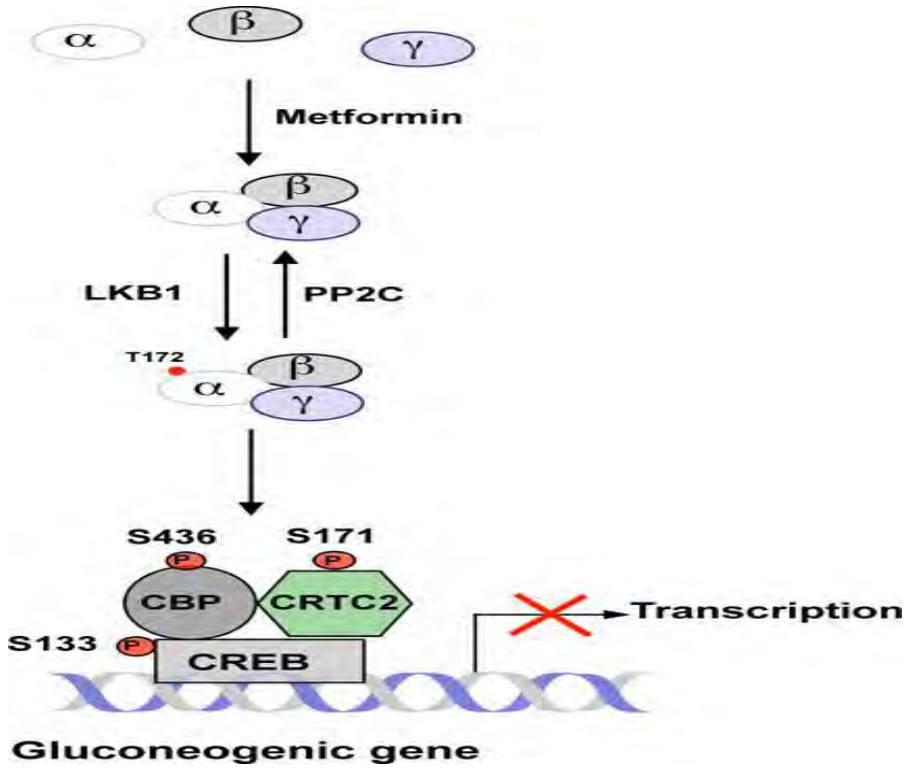

**Figure·1:**·Structure·of·Metformin·(Graham·et·al.,·2011)

## 1.5 History

In 1918 a herbal medicine in Europe found guanidine which acts as lower the sugar level in blood. In the 1920 and 1930 synthesized guanidine derivatives which also treat reducing sugar level but it was discontinued due to toxicity. In 1940 during a clinical trial it is rediscovered that it was useful to treat influenza as well as lower the glucose level in blood. In 1957 a physician of French named Jean Sterne discovered metformin which is to treat diabetes but it gets less attention because it has less potent than other biguanides like phenformin and buformin. In late 1970 this biguanides again discontinued due to high risk of lactic acidosis. Metformin was introduced to the USA in 1995 following extensive testing as its capacity to treat hyperglycemia and without causing insulin resistance or weight loss. Sixty years ago, the first metformin was used to treat diabetes but now it is the most often prescribed glucose-lowering medication globally and may have other therapeutic uses in the future (Bailey et al., 2017)

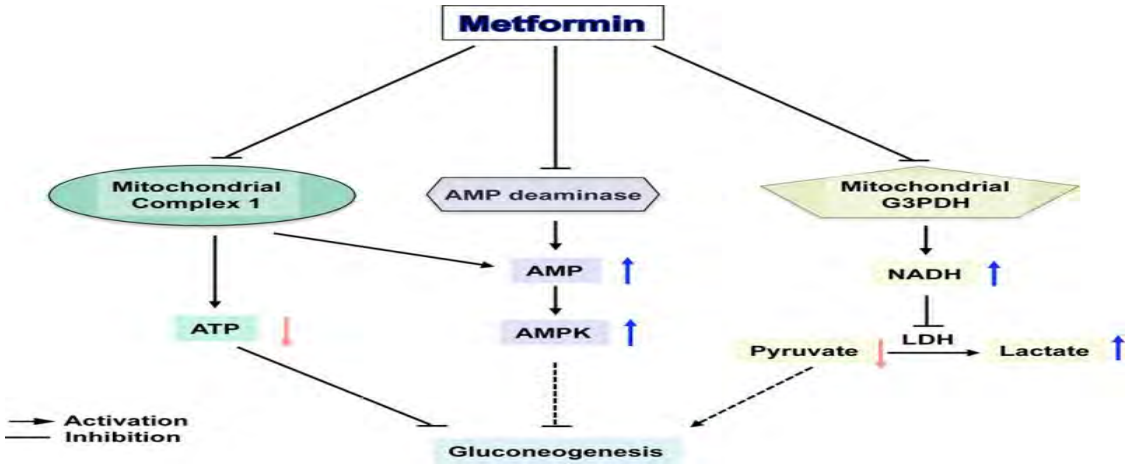
## 1.6 Mechanism of action of Metformin

Metformin primarily lowers hyperglycemia by improving insulin signaling and suppressing hepatic gluconeogenesis. But its mode of action is still unclear, particularly when it comes to how AMPK functions in conjunction with metformin and how AMPK is activated. Recent developments of the mechanism behind metformin's enhancement of insulin signaling and its inhibition of liver glucose synthesis.(An et al.,2016)




**Figure·2:**·Metformin·Blocks·Hepatic·Glucose·Formation·by·AMPKαβγ·Complex·Formatio n·(An·et·al.,·2016)

Metformin activates AMPK→ AMPKαβγ complex formation→phosphorylation of CREB→ thus inhibits transcription of gluconeogenic gene→ decrease glucose level (Fig. 2)




**Figure·3:**·Metformin·Inhibits·Gluconeogenesis·(An·et·al.,·2016)

**1. By Blocking of Mitochondrial Chain Complex 1:**

Metformin inhibits the mitochondrial chain complex 1→ thus decreases the ATP level→ inhibits gluconeogenesis.(Fig. 3)

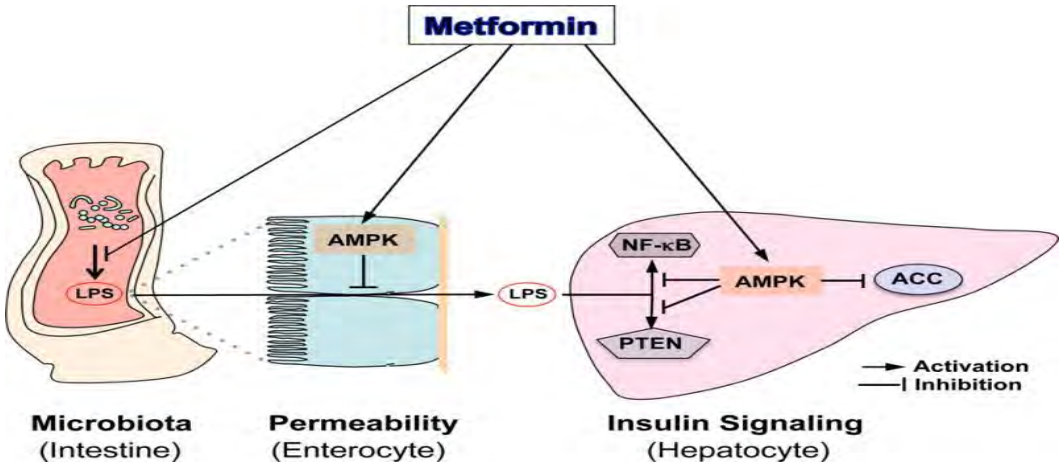
Or, metformin inhibits the mitochondrial chain complex 1→ thus increases AMP level→ resulting in the activated AMPK→ Inhibits gluconeogenesis (Fig. 3)

**2. Inhibition of AMP Deaminase:**

Metformin inhibits AMP deaminase→thus increasing AMP level→resulting in the activated AMPK→Inhibits the gluconeogenesis. (Fig. 3)

**3. By blocking G3PD:**

Metformin blocks the mitochondrial G3PD→ increases NADH level in cytoplasm→ thus inhibits the conversion from pyruvate to lactate→ inhibits LDH level→ resulting in inhibition of gluconeogenesis. (Fig. 3)




**Figure·4:**·Metformin·Activates·Insulin·Signaling (An·et·al.,·2016)

Metformin inhibits microbiota in the intestine which results in decreased LPS production. Besides, metformin changes the location across the intestinal barrier. Metformin activates

AMPK. Then AMPK inhibits LPS mediated activation by blocking NF-κB pathway and PTEN (Fig. 4)

## 1.7 Metformin acts as an Anticancer Agent

In 2001, it was observed that metformin has an anticancer effect also. It stimulates AMP that activates AMPK which is an important role for the tumor suppressor pathway. In 2005, it was first shown that metformin reduced cancer incidence in a cohort of 983 T2D patients. Recent studies show that metformin reduces the cell proliferation of cancer cells. Additionally, it inhibits the cell growth of various cancer cells such as breast cancer, prostate cancer, pancreatic cancer, colon cancer etc. Furthermore, combination of metformin with radiotherapy can reduce the tumor growth of cancer cells like breast cancer, ovarian cancer, prostate cancer in preclinical trials (Anisimov et al., 2010). Basically, metformin promotes ATM and LKB1→ activating AMPK→ inhibits mTOR→ preventing protein synthesis→ inhibits tumor growth.

According to the literature, the following mechanisms are frequently responsible for metformin's anti-carcinoma effects :

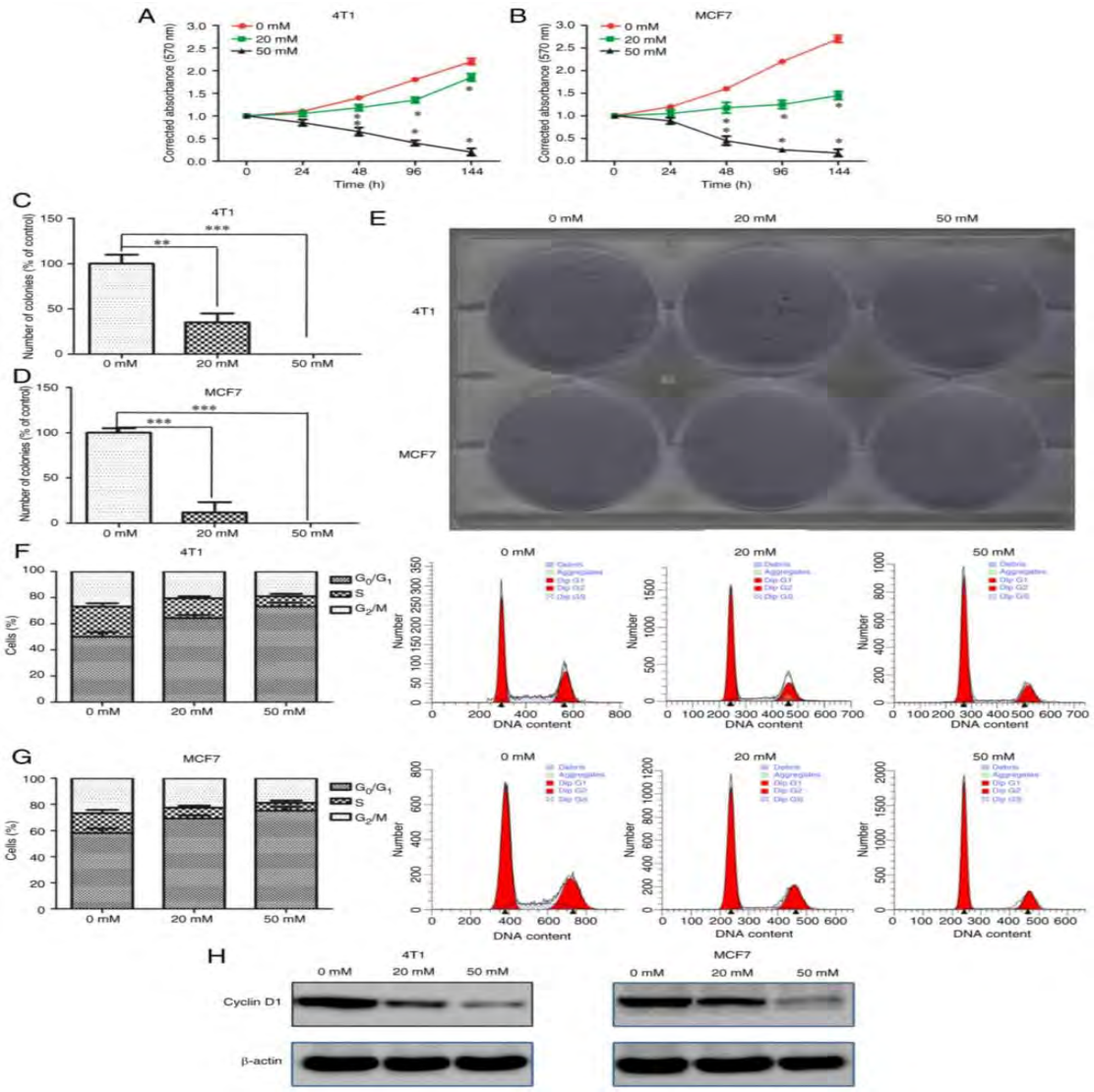
1. Activates LKB1 and AMPK→ inhibits mTOR activity,
2. Nucleic acid synthesis inhibits,
3. Inhibits G phase, S phase,
4. Activating p53 and p21 to cell apoptosis
5. Preventing Angiogenesis,
6. Protein Synthesis inhibits,
7. Prevents metastasis

# Chapter 2

# Metformin as Anticancer Agent

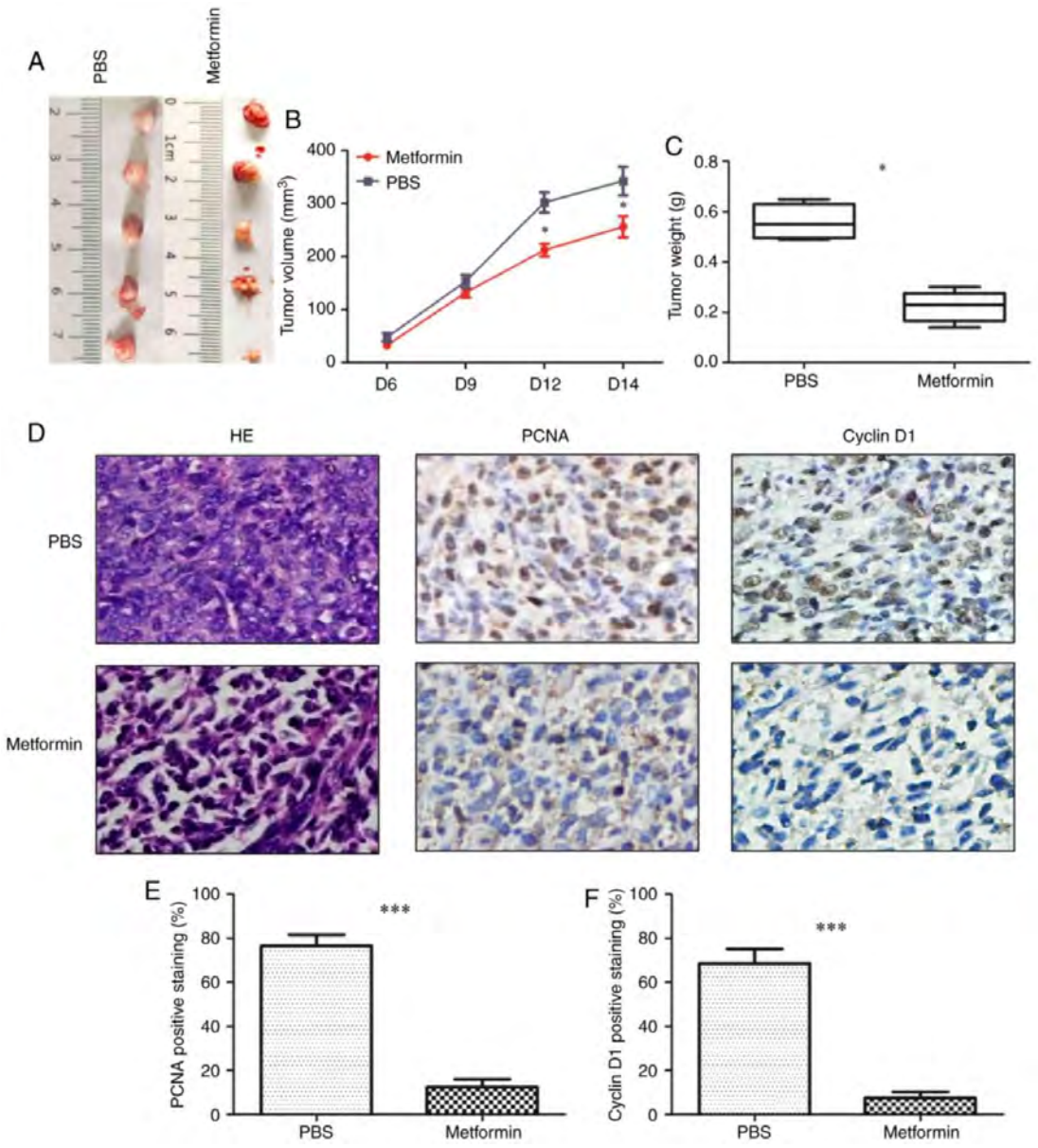
## 2.1 Metformin displays in vitro and in vivo antitumor effect against breast cancer

Preclinical and ongoing trials have indicated that metformin is an anticancer medication that can be used alone or in combination with other chemotherapy to treat breast cancer patients. Metformin reduces tumor growth in animals and reduces cell proliferation in vitro studies. In addition, it causes cyclin D1 levels down in both in vitro and in vivo→ by activated protein kinase→ blocks P-4E-BP1→ inhibits mTOR activity. Metformin inhibits cell proliferation and prevents colony formation in breast cancer cells in preclinical studies. Metformin was added to MCF7 and 4T1 cells at different concentrations (0, 20, and 50 mM) to examine its effects on cell proliferation. Cell growth was measured at the designated intervals (0-144h) in vitro. In (Fig. 5A and 5B) after 144 hours the cell proliferation of cancer cells is reduced when the drug was administered in a high concentration (50 mM). In (Fig. 5C–5E) colonies are also reduced when drugs are used in high concentration. In (Fig. 5C-5E) metformin reduced the colony formation of MCF7 and 4T1 cells by >60% compared to untreated cells. According to these findings, metformin successfully reduced breast cancer growth. G0/G1 cell cycle phase in breast cancer is inhibited by metformin. Using flow cytometry, determine the percentage of cell death in the cancer cell cycle (Figs 5F and 5G). Additionally, metformin reduced the number of cells in S phase in 4T1 cells. In(Fig. 5H) shows that metformin gives antitumor activity by inhibiting the cancer cell cycle. (Shi et al.,2021)




**Figure·5:**·Metformin·Blocks·Cell·Proliferation·and·Colony·Formation·in·In·Vitro·(Shi·et·al

.,·2021)



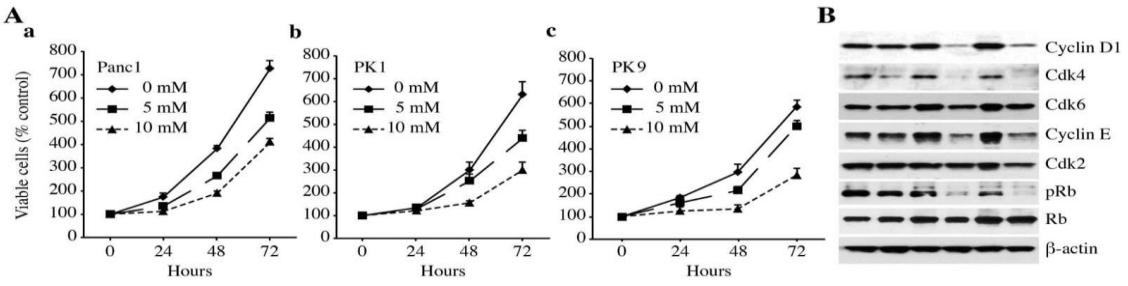

**Figure·6:**·Metformin·Blocks·Tumor·Growth·in·In·Vivo·(Shi·et·al.,·2021)

In mice metformin prevents the growth of 4T1 cell xenografts. After being injected subcutaneously into mice that reduce cell proliferation. The mice were then treated either with saline or metformin every day until the tumor size reached 4 mm. Two weeks later, the mice were sacrificed and the tumors were removed (Fig. 6A-6C). The paraffin embedded excised tumors on H&E-stained slides showed that treated mice had less tumor growth and density than untreated mice (Fig. 6D). Additionally, metformin treated tumors showed a lower expression of PCNA and cyclin D1 than untreated tumors (Fig. 6D–6F). These findings

showed that metformin prevented breast cancer cell xenografts from growing in mice (Shi et al.,2021).

## 2.2 Metformin gives In Vitro and In Vivo Antitumor Effect Against Pancreatic Cancer

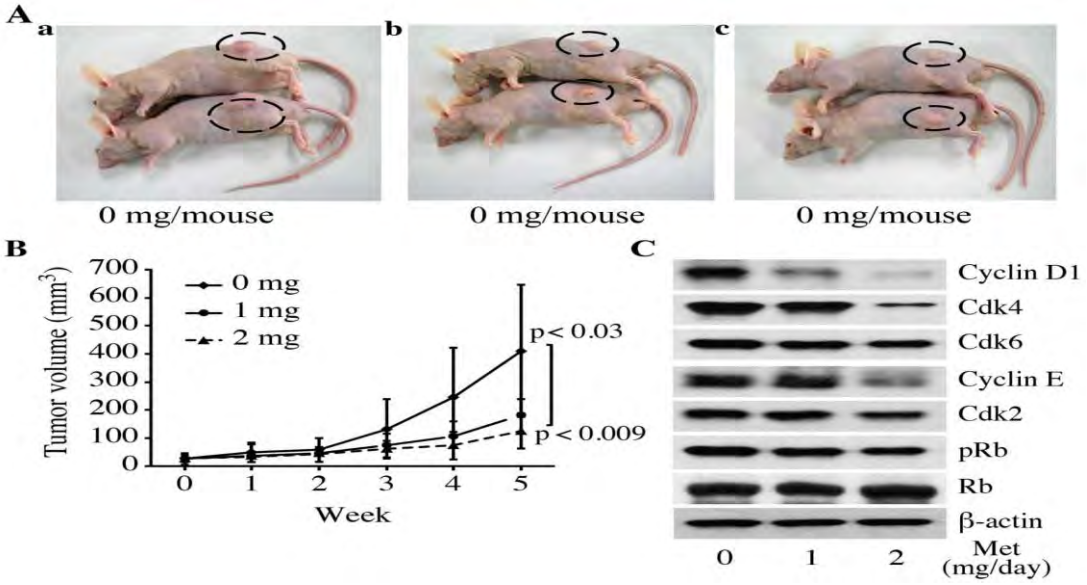
According to recent research, metformin is a popular oral biguanide family antihyperglycemic medication that may lower the risk of cancer. Metformin effects on the pancreatic cancer cell in preclinical studies. The effects of metformin on pancreatic cancer cells were investigated using the cell lines PK1, Panc1 and PK9. Metformin was administered to mice with xenograft tumors. After five weeks, the tumor's growth was noted, and the expression of proteins linked to the cell cycle was identified. In vitro proliferation of PK1, and PK9 and Panc1 cells was inhibited by metformin. The phosphorylation of retinoblastoma protein (Rb) and G1 cyclins (especially cyclin D1) were significantly reduced. Furthermore, both in vitro and in vivo metformin decreased the phosphorylation of the IGF-1R and EGFR, particularly the phosphorylation of EGFR at Tyr 845.(Kato et al.,2016)




**Figure·7:**·In·Vitro·Cancer·Cell·Proliferation·in·Pancreatic·Cancer·(Kato·et·al.,·2016)

Human pancreatic cancer cell proliferation is inhibited by metformin. The metformin effect on PK9, PK1 and Panc1 cells is to reduce cell proliferation. The cells were cultured in 10% FBS and either left untreated or subjected to 5 or 10 mM metformin. Metformin different (0, 5 and 10 mM) concentration used to reduce cell proliferation (Fig. 7A). These findings

indicate that metformin prevents pancreatic cancer cells from proliferating. The cells were either left untreated for 24 to 72 hours or treated with 10 mM concentration of metformin. The loss of the proteins Cdk4 and cyclin D1 was the most notable alteration. The levels of cyclin D1 and Cdk4 decreased at 24 hours and were completely gone at 48 and 72 hours. Metformin treated cells rapidly reduced cell proliferation comparison to untreated cells. Next, look at the cell cycle related protein levels (Cdk2, cyclin E and pRb) are treated with medication for 24-72 hours. So protein levels decreased that cause cell death compared to untreated cells (Fig. 7B).




**Figure·8:**·In·Vivo·Antitumor·Effect·on·Pancreatic·Cancer·(Kato·et·al.,·2016)

In vivo tumor proliferation is inhibited by metformin. Subcutaneously injected Panc1 cells into mice. One or two milligrams of metformin were injected intraperitoneally each day. Intraperitoneally metformin administration resulted in a significant tumor growth reduced 43.9% and 30.5% with the use of 1mg/day and 2mg/day respectively (Fig. 8A and 8B). Furthermore, 2mg metformin reduced tumor growth more compared to those treated on 1mg but metformin did not affect mice weight. Every animal used in the experiment was alive. Metformin also impacts the levels of cell cycle related proteins. In western blot analysis

determine the protein expression on the cells. The protein level drops by the use of metformin like cyclin E, Cdk2, Cdk6, pRb and Cdk4 (Fig. 8C). Based on the reduction in cell cycle protein levels that inhibit the G1 phase of cancer cells which reduced the tumor growth.

## 2.3 Clinical Trials Data of Using Metformin to Treat Cancer

In multiple animal models metformin shows the therapeutic response against cancer cells. That is why in the multiple data analysis metformin proved that it has anti tumor activity which reduces the tumor growth on multiple cancer cells like colon cancer cells, breast cancer cells, prostate cancer cells, pancreatic cancer cells etc.

**Table 1.1: Effects of metformin in various types of cancer**

| Study | Patients Number | Year | Drug | Cancer Type | Outcome |
|---|---|---|---|---|---|
| Taiwan National Insurance Data Survey Lee MS et al[71] | Total 12,005 patients and metformin use 4597 patients | 2011 | Metformin orally | Various | Reduces cancer upto 88% |
| Decensi et al[72] | Total 4042 patients and 529 cancer deaths | 2010 | Metformin orally | Various | Metformin lowering the cancer risk up to 30% |
| Bowker et al[90] | 1,000 | 2006 | metformin, sulfonylurea and Insulin | Various | Incidence of death 3.5, 4.9 and 8.8 |
| Landman et al[73] | 1,353 | 2010 | Metformin | Various | Lower the cancer risk |
| Currie et al[74] | 60,000 | 2009 | Metformin, sulfonylureas and insulin | Breast, colon, prostate and pancreas | Patients with other drugs compared to metformin has higher risk of solid cancer in future |
| Soranna et al[75] | Not provided yet | 2012 | Metformin and | Various | Reduce cancer risk |

| | | | | | |
|---|---|---|---|---|---|
| | | | sulfonylureas | | |
| Noto et al[76] | 21,195 | 2012 | Metformin | Various | Lower risk of cancer |
| Libby et al[88] | 8,000 | 2009 | Metformin | Various | Reduces Cancer risk 11.6% |
| Higurashi et al[77] | 498 | 2016 | Metformin | Colorectal adenoma or polyps | Low dose can reduce polyps and also gives a potential role of chemoprevention of colorectal cancer |
| Joshua et al[78] | 24 | 2014 | Metformin | Prostate cancer | Neoadjuvant metformin is well tolerated prostatectomy and effects on the metabolic and tissue proliferation |
| Campagnoli et al[79] | 125 | 2012 | Metformin | Breast cancer | Metformin reduce the testosterone level and insulin and its effect insulin resistance |
| Evans et al[92] | Not provided | 2005 | Metformin | Various | Metformin reduce cancer incidence |
| Monami et al[80] | 195 | 2009 | Metformin and sulfonylurea | Various | Metformin use 36 months can reduce the cancer risk |
| Home et al[81] | 105 | 2010 | Metformin and glibenclamide | Various | Lowering the cancer risk |
| Bauer et al[82] | 7,000 | 2010 | Metformin and insulin | Various | Lower prevalence of malignancy compared with other non-diabetic patient |
| Li D et al[82] | 973 | 2009 | Metformin, | Pancreatic | Metformin reduced |

| | | | | | |
|---|---|---|---|---|---|
| | | | insulin and other anti diabetic drug | cancer | the risk and insulin enhance the risk of pancreatic cancer |
| Berstein et al[84] | 90 | 2010 | Metformin, insulin and sulfonylurea | Breast cancer | Metformin has lowering the risk better compared to sulfonylureas and insulin |
| Rieken et al[85] | Total patients 6863, patient with type 2 diabetes using metformin 277 and patient with type 2 diabetes using other anti diabetic | 2014 | Metformin | Prostate cancer | RFS is better patient using metformin with prostatectomy for prostate cancer |
| Margel et al[87] | 3,837 | 2013 | Metformin | Prostate cancer | Reduced the risk of relapse and mortality |

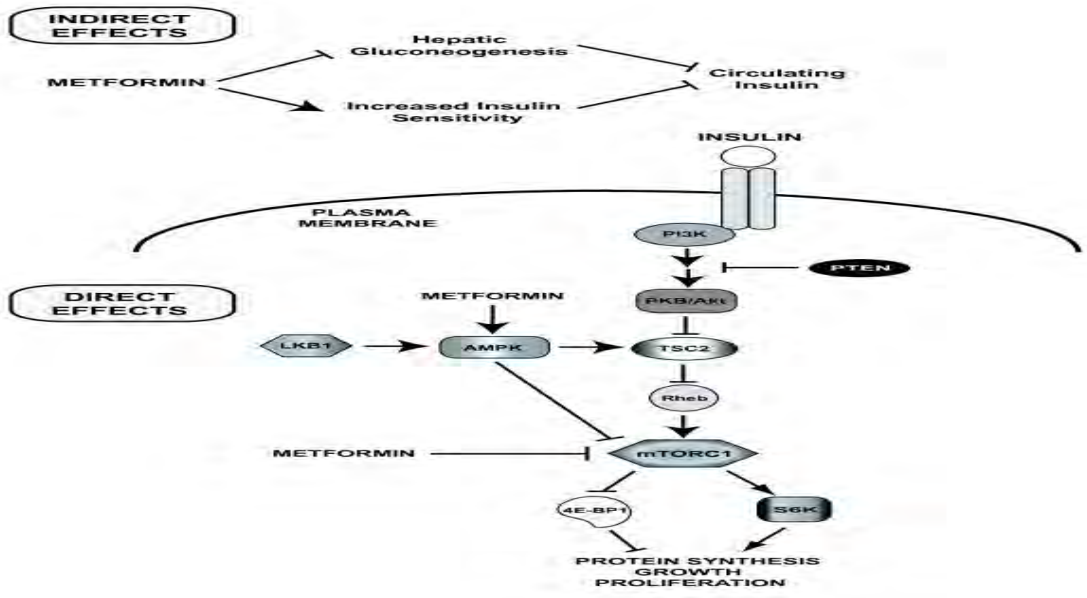
The table shows that metformin reduces the various cancer risks in patients. According to this clinical study, the use of metformin is linked to a decrease in the overall number of cancer related incidents as well as reducing tumor size and inhibiting cancer cell proliferation. Furthermore, using metformin the outcomes of multiple research studies consistently demonstrated that T2DM patients have lower risk of cancer compared to other antidiabetic drugs. In 2011 metformin was used in 4597 patients that reduced the cancer spreading up to 88%. In 2012 about 21,195 diabetic patients used metformin that reduced the risk of various cancers. In 2014 about 24 prostate cancer patients took metformin that decreased the cancer cell proliferation. In 2016 about 498 colon cancer patients were treated with metformin that inhibits the cell growth of cancer cells. According to the cases, metformin reduces cancer cell proliferation as well as cancer cell growth. Additionally, it also reduces the cancer risk.

# Chapter 3

# Proposed Mechanism of Action of Metformin as Anticancer Drug

## 3.1 Mechanism of Action of Anticancer Activity of Metformin

Metformin shows an anticancer effect of both indirect and direct acting. Direct acting associated with insulin independence. Here, metformin directly activates AMPK and blocks the mTORC1 signaling thus inhibiting protein synthesis. Additionally, AMPK activates TSC2 (tuberous sclerosis complex 2) which blocks Rheb that activates mTORC1. Then S6K activates which acts as a protein synthesis blocker. Thus inhibits the protein synthesis of cancer cells and apoptosis of cancer cells. Indirect acting associated with insulin dependence. Here, metformin blocks the transcription genes called gluconeogenesis in the liver which regulates the glucose reuptake in the muscle and inhibits the circulating glucose throughout the blood. Insulin has a mitogenic effect which is responsible for cancer cell growth. Metformin blocks the mitogenic effect to reduce the cancer cell growth (Dowling et al., 2011).




**Figure·9:**·Effect·of·Metformin·by·Indirect·and·Direct·Acting·(Dowling·et·al.,·2011)

## 3.2 Indirect Acting of Metformin as Anticancer

**1. Inhibits the Growth and Cell Proliferation:** Metformin Activates AMPK→ resulting prevents the transcription→ thus the gluconeogenic gene is not expressed→ decreasing the glycogenosis→ increasing sugar uptake in muscle cells→ decreasing the growth and cell proliferation. If insulin level is increased in blood then it needs more insulin receptors to bind in cancer cells which produce an effect called mitogenic that is responsible for cancer cell growth and cancer cell proliferation. So, block the mitogenic effect that is helpful to prevent cancer cell growth and cancer cell proliferation (Fig. 9).

## 3.3 Direct Acting of Metformin as Anticancer

**1. Inhibition of Protein Synthesis :** Inhibition of protein synthesis in three ways:

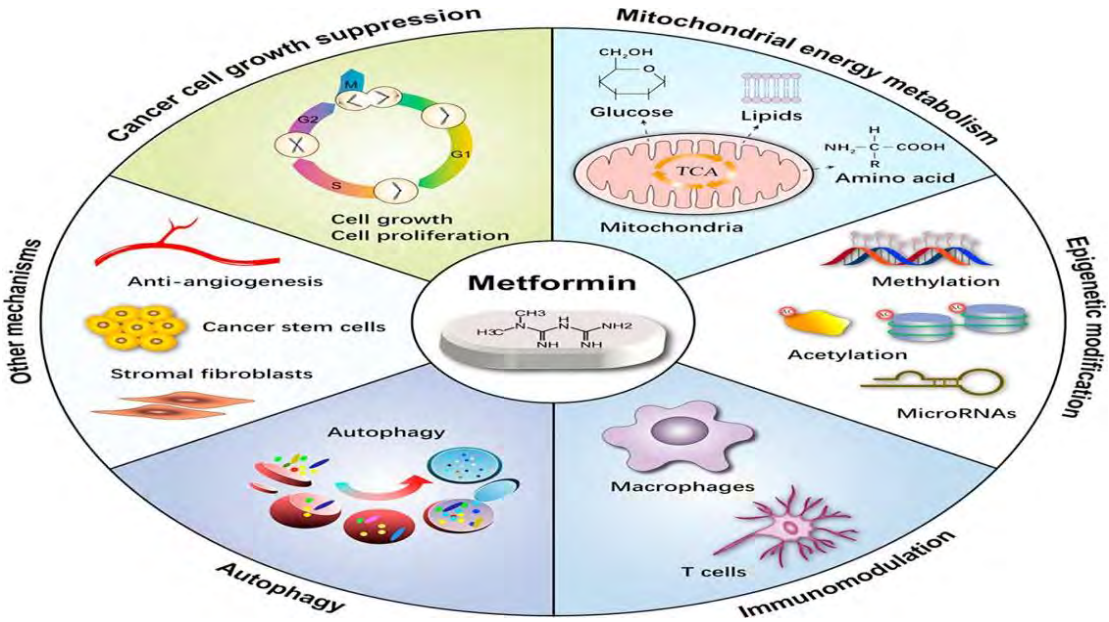
**i.** Metformin Activates AMPK→resulting AMPK prevents the mTORC1→ protein synthesis block→ cancer cell growth inhibits.

**ii.** Metformin directly blocks mTOR signaling→ protein synthesis prevents→ resulting cancer cell growth decrease.

**iii.** Metformin activates AMPK→thus stabilizes the TSC2 →inhibits the mTOR signaling→inhibits the protein synthesis→Inhibits cell growth and proliferation (Fig. 9).

## 3.4 Other Multi Action of Metformin as Anticancer

Metformin has anticancer activity as well as it has other benefits against cancer. It suppresses the tumor growth, inhibits lipid synthesis, inhibits angiogenesis, stops cancer cell growth, stops cancer cell division, induce autophagy (Zhao et al., 2020).




**Figure·10:**·Multi·Action·of·Metformin·as·Anticancer·Agent·(Zhao·et·al.,·2020)

**1. Inhibition of Lipid Synthesis:**

i. Metformin activates AMPK→inhibits ACC activity→decreases the fatty acid synthesis→Cell death. (Fig. 10)

ii. Metformin activates AMPK→thus inhibits SREBP1 in cytoplasm→decreases SREBP1 level in nucleus →decreases lipid synthesis→cell death (Fig. 10).

**2. Autophagy:**

Metformin inhibits the mitochondrial chain complex 1→thus increases AMP level→resulting in the activation of AMPK→increase P53→thus activates REDD1→inhibits mTOR→Autophagy (Fig. 10)

**3. Cell Cycle Arrest:**

Metformin inhibits the mitochondrial chain complex 1→thus increases AMP level→resulting in the activation of AMPK→increase P53→inhibits cyclin D1→Cell cycle arrest (Fig. 10)

**4. Anti angiogenic:**

Metformin inhibits the mitochondrial chain complex 1→thus increases AMP level→resulting in the activation of AMPK→AMPK converted into AMPK-P→decrease VEGF and PAI-I→inhibits Angiogenesis→inhibits cell proliferation (Fig. 10)

# Chapter 4

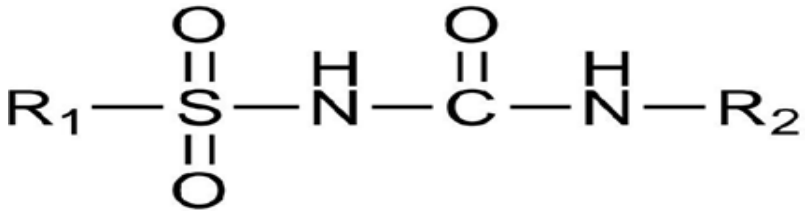
# Introduction to sulfonylurea

## 4.1 Introduction

Sulfonylureas is an Antidiabetic drug. It is used to secrete insulin in blood. It does not produce insulin but increases the secretion of insulin in blood. These are divided into three generations. First generation lowering the blood glucose level by increasing the pancreatic secretion of insulin. Second generation is more potent than the first generation. It is administered at a lower dose once a day. First generation is rarely used because it has drug drug interaction and low potency. Third generation is more potent and has less drug drug interaction (Prato et al., 2006)

## 4.2 Different Generations

The sulfonylureas are divided into three generations. Structure of sulfonylureas:

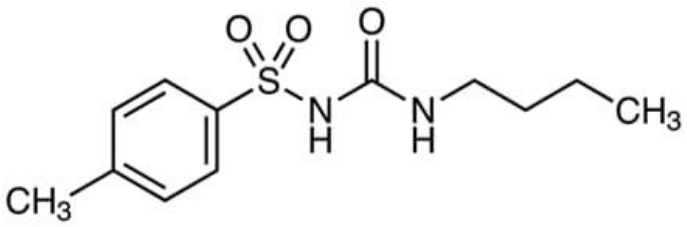



**Figure·11:**·General·Structure·of·Sulfonylureas·(Arnoldi·et·al.,·2002)

**i.** R1 must be lipophilic and has an aromatic ring with substituents. The substituents must be para position. Smaller substituents have lower potency and larger substituents gives higher potency

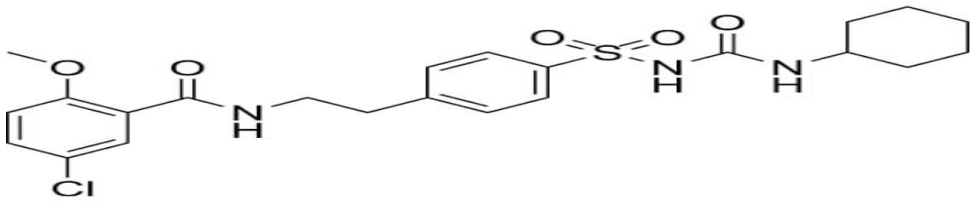
**ii.** R2 position must be lipophilic. N-methyl in R2 position is inactive. N-ethyl gives lower activity. N-dodecyl and above are also inactive. N-propyl to N-hexyl gives the most potency.

**1st Gen of Sulfonylureas:** R1 and R2 position small substitution so it has low poten



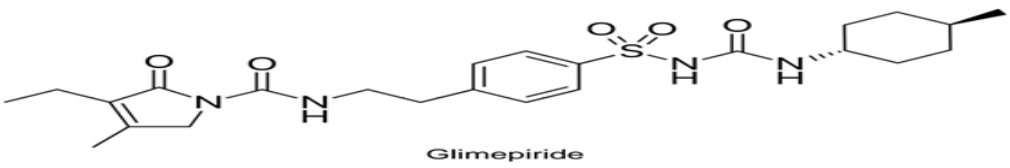
**Figure·12:**·Structure·of·Tolbutamide·(Nath·et·al.,·2010)

**2nd Gen of Sulfonylureas:** R1 and R2 have larger substituents so are more potent than 1st gen.



**Figure·13:**·Structure·of·Glibenclamide·(Nath·et·al.,·2010)

**3rd Gen of Sulfonylureas:** R1 and R2 have larger substituents than 1st and 2nd gen. So it gives higher potency than 1st and 2nd gen.




**Figure·14:**·Structure·of·Glimepiride·(Nath·et·al.,·2010)

## 4.3 History

In 1942 sulfonylureas were first discovered by Marcel Janbon. He worked on the sulfonamide antibiotics and discovered sulfonylurea which lowered the sugar level in animals. In 1960 various types of sulfonylureas were discovered and they were divided into two subtypes. First generation and second generation (Derosa et al., 2015)

**Table 1.2: Dose, Duration of action, metabolic activity and elimination pathway of different generation sulfonylureas**

| Molecules | Generation | Dose (mg) | Duration of Action (T1/2) | Activity of Metabolites | Elimination |
|---|---|---|---|---|---|
| **Tolbutamide** | I | 500-2000 | 4.5-6.5 hr (Short) | Inactive | 100% by urination |
| **Glibenclamide** | II | 2.5-15 | 5-7 hr (Intermediate to long) | 10 hr (Active) | 50% by bile |
| **Glimepiride** | II | 1-6 | 5-8 hr (Intermediate) | 3-6 hr (Active) | 80% by urination |
| **Glipizide** | II | 2.5-20 | 2-4 hr (Short to intermediate) | Inactive | 70% by urination |
| **Gliclazide** | II | 40-320 | 10 hr (Intermediate) | Inactive | 65% by urination |
| **Gliquidone** | II | 15-180 | 3-4 hr (Short to intermediate) | Inactive | 95% by bile |

## 4.4. Mechanism of Action of Sulfonylureas

Sulfonylureas binds to the sulfonylureas receptor which is present on the pancreatic β-cells. So the K ion channel close to the k ion can not release from the cell. The depolarization occurs. Thus resulting Ca channel open and Ca ion enter to the cells. Additionally increased

the Ca level in cells thus pushes the insulin secretion from cell to blood and reduces the blood sugar level.

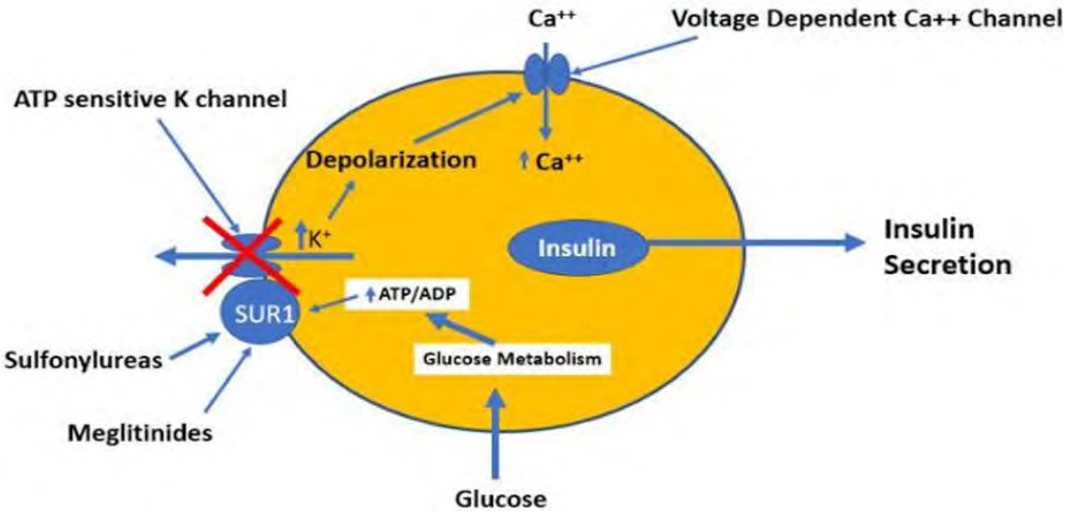



**Figure 15:** Mechanism of Action of Sulfonylureas (Kenneth et al., 2022)

# Chapter 5

# Sulfonylureas as anticancer Agent

## 5.1 Potential Anticancer Effect of Sulfonylureas

Sulfonylureas block VEGF both in vitro and in vivo which may cause vasculogenesis and angiogenesis. Additionally, it degrades the extracellular matrix which blocks MMPs resulting in cancer cell death.

**i.** Sulfonylureas inhibit VEGF → prevent vasculogenesis so no new blood vessels are formed → also prevent angiogenesis so inhibit the own blood supply→ cancer cell death.

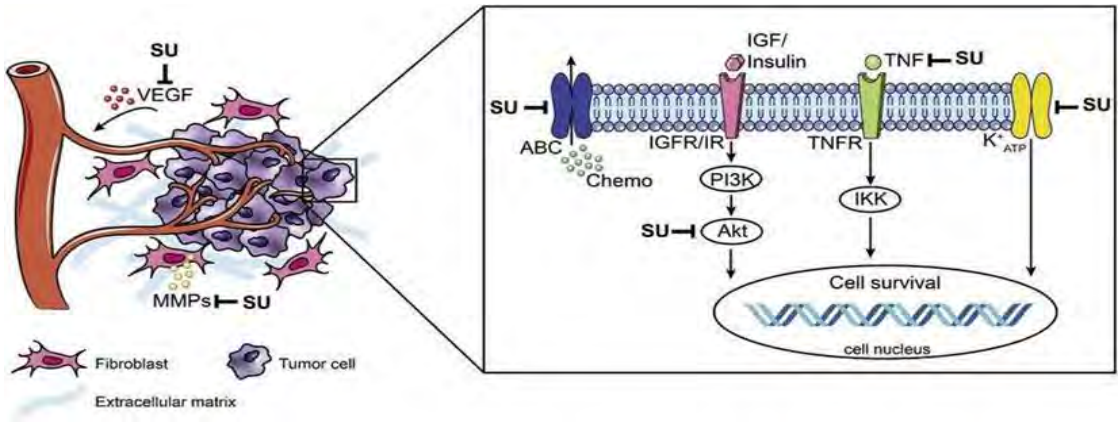
**ii.** Sulfonylureas block MMPs ( matrix metalloproteinases are an important role of cell proliferation) → resulting stop cell proliferation → cause death of cancer cells.

**iii.** Sulfonylureas block the ABC transporter → so the chemotherapeutics cannot efflux to the cell→ increase the chemotherapeutics in the cell→ cure the cancer cells.

**iv.** Sulfonylureas block Akt→ cell growth inhibit → prevent cancer cell survival.

**v.** Sulfonylureas block TNF→ prevent the binding of TNFR→ decrease the TNF levels→ resulting pro cell death of cancer cells.

**vi.** Sulfonylureas block K+ ion channels→ depolarization occurs→ prevent cell growth → cancer cell apoptosis (Fig. 16).




**Figure·16:**·Potential·Anti·Cancer·Effect·of·Sulfonylureas·(Hendriks·et·al.,·2019)

## 5.2 Sulfonylureas gives Anticancer Effect on In Vitro and In Vivo

Diabetic patients are highly prone to cancer specially the patients associated with type 2 diabetic or diabetes mellitus. In type 2 diabetes, the patient suffers from overactivation of the signaling pathway of insulin growth factor 1 and the excessive secretion of insulin (Hyperinsulinemia). In hyperinsulinemic conditions, patients' bodies become resistant to insulin and body cells do not respond to insulin properly. To suppress the situation, the body produces more and more insulin and therefore the patient is suffering from the condition called hyperinsulinemia. Patients' blood insulin levels become much higher than the accepted range and eventually lead the condition to cancer. The study report that is found for the sulfonylureas is less reliable and consistent. However, glibenclamide which is a potassium channel blocker, produces ROS (reactive oxygen species), causes formation of free radicals and kills the cancer cells. Furthermore the diaryl sulfonylureas (DSU) is showing pro apoptotic activity against the cancer cells which have multiple drug resistance.

In the in vitro and In vivo clinical trials few drugs of sulfonylureas groups are actively working against cancer cells.

**Glibenclamide:** Glibenclamide acts as a drug which can successfully inhibit the growth of cancer cells by inhibiting the cancer growth inhibitor cells. Glibenclamide inhibits the ATP

sensitive potassium channel and allows the calcium influx inside the cells which eventually leads to the exocytosis of insulin. Glibenclamide works to inhibit the wide variety of cancer including human prostate cancer, hepatic cell line cancer, human breast cancer by inhibiting G0/G1 arrest or ATP sensitive potassium channel inhibition, bladder tumor cancer by inhibiting membrane gated potassium channel, ovarian cancer, carcinoma cells adhesion and proliferation inhibition and colon cancer. In 2008, Glibenclamide first worked as an anticancer agent to treat gastric cell line cancer. In clinical trials, it is observed that the cell line caspase activity increases and the biphasic, epithelioid and sarcomatoid activity is increased significantly. Furthermore, the ROS that is produced by the drug is not produced in higher levels .For this reason the toxicity related to build up the reactive free oxygen io is very less. After analyzing the in vitro and in vivo result of glibenclamide we can summarize that,

**1.** Glibenclamide intrigue cell mediated primary apoptosis,

**2.** It Sensitizes the malignant pleural mesothelioma cell lines, which lead to apoptosis.

**3.** Through other unknown mechanisms like epithelioid and sarcomatoid histotypes mechanism.

**4.** Activation of ER stress induced caspase 10 and other effector caspases 3 and 7

5. and lastly, decrease the secretion of different pro angiogenic proteins.

**Glipizide and Glimepiride:** These two antidiabetic drugs have no anticancer effects. No clinical data, evidence or in vitro and in vivo report has been found.

**Diaryl sulfonylureas (DSU):** DSU are structurally similar to glibenclamide and they are effective against solid tumor, haematologic cancer. In the 1980s, an In vivo and in vitro

report of DSU was published which shows that it is effective against cancer. Its mechanism is also similar to glibenclamide. Preclinical studies show that it is a highly protein bound drug which has a longer half life and it does not work like other conventional chemotherapeutic effects. Phase I and phase II study has been performed over this drug in the treatment of ovarian cancer, pediatric malignant solid tumor and conventional solid tumor. After gaining good and satisfactory results in phase I and Phase II the further research has been conducted to determine the toxicity profile and approximate dose for the drug. Sulofenur 800mg/m2 dose show response in 42% patients and it shows partial response within 6.5 week to 18 week in around 15% of the patient. Major toxicities along with decreased RBC count, methemoglobinemia and severe anemia were reported in this Phase I and Phase II trials. Toxicity was the main concern and the doses were tested in phase II trials after 5 days treatment and 2 days rest in a week and this is continued for 3 weeks to reduce toxicity. After doing the resting days, still the dose reduction is required for almost 31% patients.

In order to bypass the toxicities, new drug molecule searching is carried out which includes no formation of toxic anneline metabolite formation. This metabolite causes the toxicity of this drug molecule in cancer treatment. The strategy shows that 1000 mg/m2 dose daily needs to be administered weekly for 3-4 weeks and it shows reduced toxicity. However, It shows hematologic toxicities, thrombopenia and neutropenia.

More recently, newer synthesized derivatives DW2282 methanesulfonates and arylsulfonamide were successfully produced which shows promising anticancer effects. It acts as common chemotherapeutic drugs taxanes and vinca alkaloid and inhibit tubulin polymerization, target MDR tumor and inhibit G2/M phase of cell cycle.

## 5.3 Clinical Studies of Sulfonylurea for Lowering the Cancer Risks

Patients with T2DM have higher cancer risk compared to other patients. T2DM patients have a higher risk of insulin resistance and hyperglycemia which is associated with cancer development. Patients are follow up the drugs from 4.8 to 14 years where five to six studies are focused on cancer incidence and other studies are focus on another site of cancer incidence (Bo et al., 2013; Chang et al., 2012; Monami et al., 2007; Tuccori et al., 2015; Yang et al., 2010a; Monami et al., 2009).

**Table 1.3: Clinical Studies of Sulfonylurea for lowering the cancer risks**

| Author | Design | Duration | Number of patients | Sulfonylureas | Comparator | Outcome |
|---|---|---|---|---|---|---|
| Bo et al., 2013 | R | 14 years | 1277 | Gliclazide | Glibenclamide | Hazard ratio 0.30 (0.16-0.55) ( Mortality) |
| Bo et al., 2013 | R | 14 years | 1277 | Tolbutamide | Glibenclamide | Hazard ratio 0.48 (0.29-079) (Mortality) |
| Monami et al., 2009 | R | 5 years | 568 | Glibenclamide | Gliclazide | Odds ratio 3.6 (1.1-11.9) (Mortality) |
| Tuccori et al., 2015 | P | 5.3 years | 52,600 | Glibenclamide | Another 2nd generation sulfonylureas | Hazard ratio 1.09 (0.98-1.22) (Cancer risk) |
| Tuccori et al., 2015 | P | 5.3 years | 52,600 | Glibenclamide | Another 2nd generation sulfonylureas | Hazard ratio 1.27 (1.06-1.51) (Cancer risk) |
| Chang et al., 2012 | R | 7.4 years | 108,920 | Sulfonylureas 1st or 2nd generation | No use of sulfonylureas | Odds ratio 1.08 (1.01-1.15) (Cancer risk) |
| Chang et | R | 7.4 years | 108,920 | Glimepiride | No use of | Odds ratio 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| al., 2012 | | | | | sulfonylureas | (0.93-1.08) (Cancer risk) |
| Yang et al., 2010a | P | 4.8 years | 6103 | Glibenclamide | Never use of Glibenclamide | Hazard ratio 0.67 (0.51-0.89) (Cancer risk) |
| Yang et al., 2010a | P | 4.8 years | 6103 | Gliclazide | Never use of Gliclazide | Hazard ratio 0.65 (0.49-0.83) (Cancer risk) |
| Monami et al., 2009 | P | 6.5 years | 390 | Glibenclamide | Not use of Glibenclamide | Odds ratio 2.24 (1.21-4.14) (Cancer risk) |
| Monami et al., 2009 | P | 6.5 years | 390 | Glibenclamide | Not use of Glibenclamide | Odds ratio 2.62 (1.26-5.42) (Cancer risk) |
| Monami et al., 2009 | P | 6.5 years | 390 | Gliclazide | Never Gliclazide | Odds ratio 0.39 (0.21-0.74) (Cancer risk) |
| Monami et al., 2009 | P | 6.5 years | 390 | Gliclazide | Never Gliclazide | Odds ratio 0.40 (0.23-0.69) (Cancer risk) |

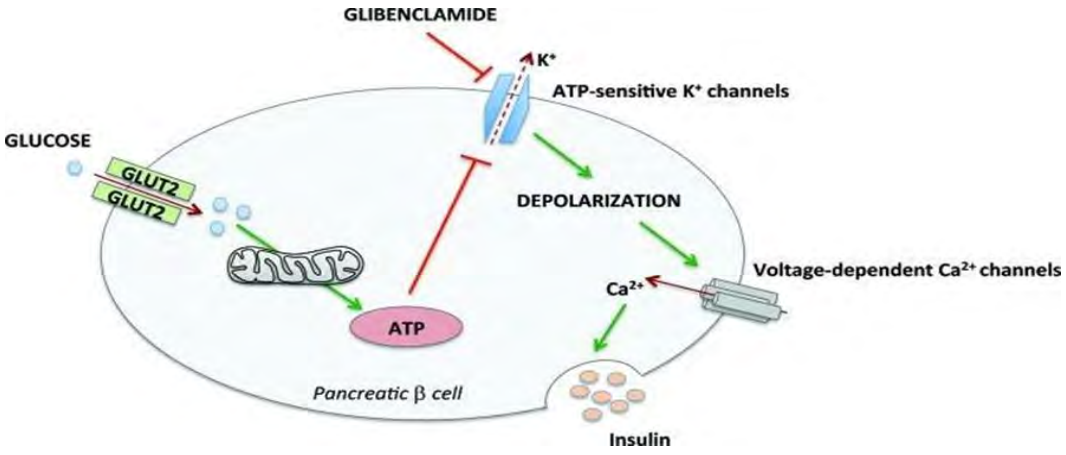
The table shows that in 2013, 1275 patients used tolbutamide over 14 years which lowered the cancer risk up to 0.29 to 0.79%. Another study published in 2013, using gliclazide over 14 years around 1277 patients, can also lower the cancer risk 0.16 to 0.55%. In 2015 Glibenclamide was used by around 52,600 patients over 5 years. It can lower the risk of cancer up to 0.98 to 1.22% compared to other 2nd generation sulfonylureas. In 2007 Glibenclamide used around 568 diabetic patients over 5 yrs it can reduce the cancer risk 1.1 to 1.9%. In 2012 glimepiride used around 108,900 patients over 7 years. It can reduce the risk of cancer up to 0.93 to 1.08%. All the studies found that sulfonylureas did not enhance the cancer risk. The sulfonylureas monotherapy reduces the cancer risk compared to other antidiabetic drugs.

# Chapter 6

# Mechanism of Action of Sulfonylureas as Anticancer Drug

## 6.1 Mechanism of Sulfonylureas as Anticancer Drug

The preclinical and clinical studies show that it has anti tumor activity. It can suppress tumor growth and also inhibit the cell proliferation of cancer cells. Additionally, it can inhibit metastasis as well as angiogenesis. Recent studies show that the second generation is more potent than the 1st gen. It has more antitumor effects than the 1st one (Pasello et al., 2013).




**Figure·17:**·Pancreatic·Cells·Secrete·the·Insulin·by·using·Glibenclamide·(Pasello·et·al.,·2013)

Glibenclamide is a 2nd gen of sulfonylureas drug which is used in diabetes patients. The drug binds to the SUR1 receptor that is present on the pancreatic cells. It blocks the ATP synthesis K ion channels. So depolarization occurs in the cell. Resulting voltage gated Ca ion channel opens and Ca ion enters the cell. So Ca level increases in the cell that can cause insulin secretion.

## 6.2 Antitumor activity of Glibenclamide

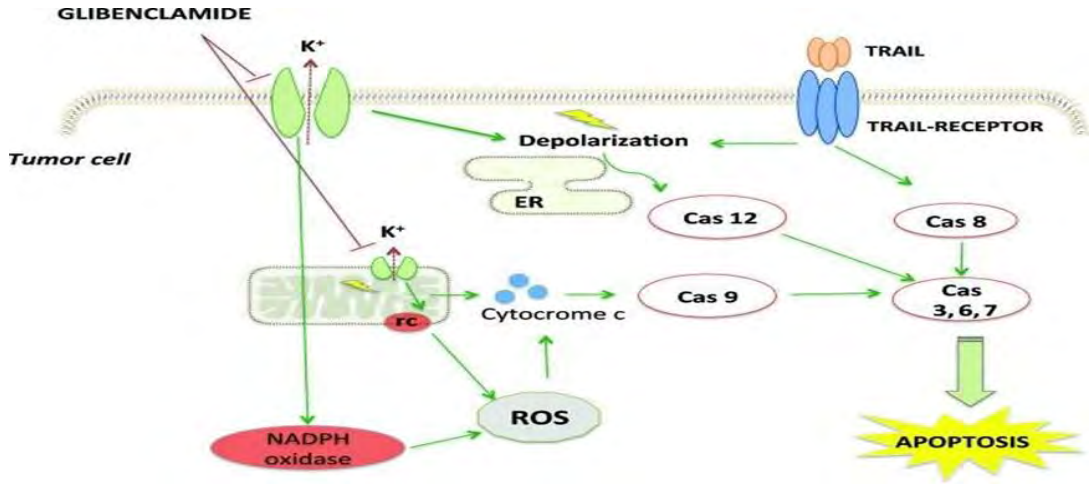
Glibenclamide has antitumor activity because it enhances NADPH oxidase and ROS formation which helps to activate the caspase thus resulting in tumor cell death.

**i.** Glibenclamide blocks k ion channels→ depolarization occurs→ thus activated caspase 12 → caspase 12 activated cas 3,6,7→ resulting tumor cell apoptosis (Fig. 18).

**ii.** Glibenclamide blocks mitochondrial k ion channels→ production of cytochrome C→ activates caspase 9→ thus promotes cas 3,6,7→ tumor cell apoptosis (Fig. 18).

**iii.** Glibenclamide prevents K ion channel→ increase the NADPH oxidase→ production of ROS→ Cytochrome C release→ activating cas 9→ thus activating caspase 3,6,7→ apoptosis of tumor cell (Fig. 18).

**iv.** Glibenclamide inhibits k ion channels in mitochondrial membrane→ production of ROS→ cytochrome C release→ activating caspase 9→ activates cas 3,6,7→ apoptosis of tumor cells (Fig. 18).




**Figure·18:**·Proposed·Model·of·Antitumor·Activity·of·Glibenclamide·(Pasello·et·al.,·2013)

# Chapter 7

# Conclusion

In cancer patients antidiabetic drugs metformin and sulfonylureas reduce the cancer risk. In preclinical and clinical studies show that it has anticancer activity which inhibits the cancer risk. Metformin inhibits the nucleic acid synthesis as well as protein synthesis so it reduces the tumor growth as well as inhibits the cell proliferation and apoptosis occurs. On the other hand, sulfonylureas block the K+ ion channel thus depolarization occurs and activating the caspase 3,6,7 thus can cause cancer cell apoptosis. It also inhibits the angiogenesis and metastasis so it destroys the blood supply of tumor cells and cancer cells do not spread in the other part of the body. In 2014 a study showed that about 347 million people have type 2 diabetes worldwide and have increased the cancer risk of those patients who have type 2 diabetes. The observation shows that the highest insulin concentration in the blood of those patients may cause mitogenic effects so this increases the higher risk of cancer in those diabetes patients. So antidiabetic drugs are used which have to control insulin secretion as well as anticancer effect. The clinical studies of both metformin and sulfonylureas shows that the monotherapy of metformin as well as sulfonylureas have lowered the cancer risk. Their consistent outcomes also reduce the cancer risk factor. Additionally, in vitro and in vivo studies provide that metformin has an antineoplastic effect and also it has a direct and indirect effect which lowers the glucose concentration in the blood. Besides, it activates liver enzyme AMPK which suppresses cancer development. All the clinical studies provide that sulfonylureas reduces the cancer risk. The second generation sulfonylureas gliclazide has lower cancer risk compared to the glibenclamide users. Glibenclamide has prolonged hypoglycemia risk compared to metformin and other sulfonylureas. Besides, glibenclamide not only binds to the SUR1 which is located in the pancreatic β cells but also SUR2A and

SUR2B which are located in the cardiac muscle. On the other hand, gliclazide is more selective to SUR1. Higher insulin levels enhance the tumor growth in vitro and in vivo. Metformin prevents insulin resistance thus lowering the insulin and glucose level in the blood as well. Overall, this review article is significant as it examines a much need knowledge gap in the literature of investigating the anticancer effects of metformin and sulfonyl ureas, drugs which are traditionally used in treatment of diabetes mellitus. It is expected that this work may positive impact future researchers entering in these lines of studies.

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