Journal of Pharmaceutical Research International

33(50A): 170-183, 2021; Article no.JPRI.76134
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Mechanism of Anticancer Activity of Compounds Isolated from Two Species of Ziziphus (*Z. jujube* and *Z. mauritiana*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i50A33393
Editor(s):
(1) Dr. Begum Rokeya, Bangladesh University of Health Sciences, Bangladesh.
Reviewers:
(1) Amr Nassrallah, Cairo University, Egypt.
(2) Timothy Omara, Moi University, Kenya.
Complete Peer review History: https://www.sdiarticle4.com/review-history/76134

Review Article

Received 02 September 2021
Accepted 11 November 2021
Published 16 November 2021

ABSTRACT

Medicinal plants have been used to treat diseases for centuries. One group of such plants is *Ziziphus* species belonging to Rhamnaceae family. The extracts from plants of this genus has been found beneficial for the treatment of cancer caused by high production of reactive oxygen species resulting from different oxidative stress mediated conditions. The mechanism of anticancer activity of two different species of this plant (*Z.jujube* and *Z.mauritiana*) have been discussed in this review. The constituents of this plant include the flavonoids, triterpenes, potassium, calcium, phosphorus, iron, zinc, copper and polysaccharides such as reducing and non-reducing sugars. The underlying mechanisms of both species include the (Tumor protein P53) P53, (signal transducer and activator of transcription) STAT, (Matrix metalloproteinases) MMPs, (clustered regularly interspaced short palindromic repeats) CRISPR and flavonoids and triterpenic acid mechanisms. The effects of the extract on different cells lines in both *in vitro* and *in vivo* models have been studied by observing the induction of apoptosis and reduction in angiogenesis leading to reduction in progression and proliferation of cancer cell lines. The biological properties of Ziziphus include the anti-inflammatory, antioxidant, anticancer and hepato-protective characteristics.

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Keywords: Ziziphus; antitumor components; molecular targets; oxidative stress; Ziziphus jujube; Ziziphus mauritiana.

1. INTRODUCTION

Jujuba plant belongs to Rhamnaceae family with the history of about 4000 years being native to Chinese medicine. It is also a part of Iranian traditional medicine as it possesses laxative and blood purification characteristics [1]. Many major minerals such as potassium, calcium and phosphorus and some other minerals such as iron, copper and zinc are also a part of its fruits composition [2]. Different vitamins including vitamin B and C are also present in great amounts. The main location of plant includes Meditarranean, Asia and America regions (tropical and subtropical). As this plant is native to china, this country is responsible for 90% production of jujuba in the world. This plant is used in the treatment of many diseases such as diabetes, skin diseases, liver infections, cancer and for purification of blood also. Different types of fruits and vegetables possess chemo-protective effects towards the damage caused by anticancer drugs to organs and tissues. Many plants comprise natural and bioactive compounds which can be used in the development of new drugs such as anticancer drugs [3]. The extract of jujuba plant consists of amino acids, proteins and many fatty acids inclusive of oleic, linoleic, palmitic and palmitoleic acids. This plant also contains dietary fibers with some fructose contents which regulate blood sugar levels. Different kinds of sugars including glucose, sucrose, sorbitol and rhamnose are also the part of its nutritional composition. The nutritional composition of this fruit also comprises of phenolic compounds which makes it to be used in the treatment of different diseases [4]. Phenolic acids and flavonoids are examples of phenolic compounds. Triterpenic acids holds much importance in research fields because of having anti-microbial, anti-oxidant, anti-inflammatory and hepato-protective characteristics. Different types of triterpenic acids such as betulinic acid (BA), oleanolic acid (OA) and ursolic acid (UA) also possess cytotoxic effects against different types of cancer. The induction of apoptosis is the major mechanism of bioactive compounds with anticancer properties [5]. The anticancer effects of jujuba plant were studied on human hepatocellular carcinoma cells and the experiments were performed on HepG2 cell lines [6]. The study showed that these three acids (BA, OA, and UA) induced apoptosis and markedly reduced cell viability in HepG2 cell line. Two different cellular signaling pathways including PI3K and MAPK are predicted to be involved in the initiation of apoptotic effect of triterpenic acids [6]. This effect is followed by the activation of caspases such as caspase-3 and caspase-9. These two also participated in the stimulation of apoptosis. BA stimulated the expression of BCL-2 protein family of anti-apoptotic genes. Not only these genes but also the family of pro-apoptotic genes is also involved in this mechanism. Two other cellular signaling pathways are involved in the induction of apoptosis by the concern of mitochondria in aid with BA mode of action. These two pathways include extrinsic pathway (also known as receptor pathway) and intrinsic pathway (also known as mitochondrial pathway). Another mechanism of BA involves the activation of inflammatory pathway with the assembly of ROS (reactive oxygen species) [7]. Angiogenesis can be repressed by betulinic acid by the declined expression of an enzyme. The extract of jujuba plant also consists of de-protienized polysaccharides, have anti-proliferative ability, which can aid in the induction of apoptosis by increasing the expression of caspase-3 and caspase-9. Chemo-resistance in cancer cells result due to dysfunction of anti-apoptotic and pro-apoptotic pathways [8].

In this review, the anticancer properties of two different species of jujube plant have been discussed with their components and underlying mechanisms to study the effects of extract of plant. Both species of jujube plant are commonly known as ber, one is known as Chinese ber or red date (*Ziziphus jujuba*) and the other one as Indian ber (*Ziziphus mauritiana*). Both of these species are also cultivated in Pakistan and commonly consumed as well because their fruits are edible. Immune responses can be reduced or stimulated by the use of medicinal plants in order to treat immunological diseases [9]. The oxidative stress is considered to be responsible for causing a number of diseases characterized by high production of reactive oxygen species, which can be treated or reduced by the anti-oxidant, anti-inflammatory and anti-tumor activities of different species of Ziziphus plant [10]. The pathways involved in anticancer activities of both species of jujube plant have been discussed in this review and could offer insights on the development of anticancer agents

2. BOTANY, MEDICINAL USES, PHYTOCHEMISTRY ANDANTICANCER/ANTITUMOR EFFECTS OF *Z. jujuba*

2.1 Botany

Natural products including plants and vegetables have been used as primary source for the development of medicine to treat different diseases since prehistoric period. Plenty of medicinal plants are present in the world. One of them is *Z. jujuba*. This plant belongs to Rhamnaceae family. This plant has 40 species with beneficial uses distributed worldwide but only 4 are used for medicinal purposes. This plant is used for development of medicines in many countries including China, India and many Asian countries also [11]. Its leaves are green and flowers are yellowish in color. As this plant is native to China, it is considered as one of the important medicinal fruit in Chinese traditional medicines [12].

The fruit color is green when immature and after ripening the fruit color turns out to be red. This plant is acidic in taste. The fruits, roots, seeds and leaves of this plant are used for medicinal purposes [13]. The bark of this plant is also used to cure diseases. This plant is traditionally used for medicinal purposes since centuries. This plant holds much importance worldwide because of its medicinal benefits including pharmacological characteristics and that is why the cultivation of different species of plant takes place in many countries of the world. The plant is known by many names. Recent studies about the plant show that the origin of this plant is Africa and Australia. The nutritional composition of plant consists of amino acids, proteins, vitamins, carbohydrates, oil and phenolic compounds [14]. Other elements which are present in this specie of plant include calcium, phosphorus, iron, glycosides, flavonoids and terpenoids. Bioactive compounds such as oleic acid and palmitic acid is also present with secondary metabolites [15].

Recent studies show that this plant also has the ability to hold back the aging progression in women. This plant can also be used to treat different types of ulcer such as rectal and intestinal cancer. The healthy components of this plant's fruit include vitamin C, phenolic compounds, terpenoids and polysaccharides [2]. The fruit of this plant has also shown to be a rich source of vitamins. Different parts of this plant possess different abilities such as leaves having therapeutic properties, seeds with oil having anti-microbial and fruit having anti-bacterial and anti-fungal abilities respectively.

Some countries use the powder form of the stem, bark and leaves of this plant for the treatment of wounds. As this plant have shown to be the rich source of polyphenolic compounds, it is essential for diabetic patients in order to control the diabetes. The height of this plant tree varies regarding the location. In some countries its 6-9 m high whereas in other countries its 9-12 m high. Their trees are more branched than others. The fruit size of this plant is also different regarding the cultivation and environmental conditions [16].

2.2 Phytochemical and Mineral Composition of *Z. jujuba* Plant

Recent studies show that this plant reduces the level of glucose and lipids in blood leading to a turn down in LDL and cholesterol, due to which it can used to control the blood pressure in hypertensive patients. The fresh fruit of jujube tends to contain more vitamin C as compared to dried fruit, so it's essential to eat the fresh fruit of this plant. Fatty acids also make a part of nutritional composition of this plant. The leaves of this plant contain more flavonoids than the fruit. *A saponin* in the form of jujubogenin can be used to control the cancer [17].

Terpenoids in the form of triterpenic acids have been found in the fruit of jujube plant. The stem bark contains betulinic acid (BA) which tends to reduce the metastasis in infection caused by HIV. The betulinic acid has also been shown to possess the anti-genotxic activity which means that it can reduce the breakdown of DNA caused by alkaloid agents. This study showed the effects of anti-genotoxic activity with better results with ethanol fraction of jujube plant extract in comparison to other fractions. The results of this study proved that BA could reduce the damage to DNA [18].

Neuro-protective abilities are also present in the fruit of jujube plant. The flavonoids present in the fruit of this plant can tranquilize the mind and enhance the sleeping time by the brain. So this plant can also be used to treat the brain diseases and disorders related to sleep such as insomnia and Alzheimer disease. The water fraction of jujube extract can also be used for the treatment of neurological diseases as shown by the recent

studies and research [19]. The immature jujube fruit, green in color, tends to contain more phenolic compounds, biological active components and characteristics in comparison to mature or ripe fruit, which is red in color [20].

Glucose homeostasis is largely maintained by skeletal muscles. Glucose up take by skeletal muscles is up to 70%, which is further increased by insulin and exercise. The activity of insulin of regulating glucose is reduced in obese persons due to high amount of adipose tissue resulting in the release of harmful compounds such as free fatty acids and inflammatory cytokines in large amount as well. This reduction in glucose uptake leads to the onset of diabetes. This can be prevented by consuming the edible plant herbs with anti-diabetic and anti-inflammatory characteristics such as *Z.jujube* plant containing polyphenols. These polyphenols have been found in regulating the glucose uptake either by reducing or inducing its uptake in adipose tissue. The glucose transporter 4 (GLUT) is responsible for glucose uptake by adipocytes. *Z. jujube* plant also contains triterpenoids and polysaccharides in addition to polyphenols [21].

Different types of triterpenoid acids such as betulinic acid, oleanonic and ursonic acid have been to possess anti-hyperglycemic properties. An experimental study was conducted using the *Z.jujube* plant from three different countries containing these triterpenic acids to investigate its hyperglycemic properties using rat models and ethyl acetate fraction with water. The results of this study concluded that triterpenoids increased the uptake of glucose by utilizing the GLUT 4 mechanism. The methanol fraction of *jujube* plant had proposed the increase in glucose uptake. The enhanced effect on glucose uptake was shown by jujube plant from Japan in comparison to other countries (China and South Korea) because of the presence of triterpenoids in higher amount in Japanese *jujube* plant [17].

Ursonic acid (UNA) is a triterpenoid constituent of *Z.jujube* plant. Ursolic acid (ULA) is secondary metabolite produced by plants [22]. Both of these can be used for the production of anti-cancer vaccine as they possess anticancer properties of inducing apoptosis and reducing the progression of cancerous cells by the suppression of MMPs (matrix metalloproteinase) which are responsible for the metastasis in cancer cells [23]. Recent studies show that they also regulate MAPK pathway for induction of apoptosis in cancer cells. The pharmaceutical properties of UNA are due to the presence of a keto-group at C3 position [24].

Recent studies show that the jujube fruit also contains high amount of nucleosides and nucleo-bases. Jujube fruit has also been found to be a rich source of vitamin C. The jujube fruit also contains polysaccharides in the form of reducing and non-reducing sugars and some other compounds as well i.e. tocopherol and *β*-carotene. The phenolic acids present in jujube fruit include Hydroxy-benzoic acid [25]. The hepato-protective effect of jujube fruit was studied in mice with liver injury induced by CCl_4. The results of the study showed the reduced levels of AST, ALT and LDH in treated liver serum. The polysaccharides present in jujube fruit also reduced the anti-oxidant level by the reduction in ROS and inflammatory activity. Not only the fruits of jujube plant contain the medicinal nutrients but also the peel of the fruit contains the high amount of medicinal components [26].

2.3 Mechanisms of Anticancer Activity

Plants have been used as medicines since ancient times. In some countries they are traditionally used as medicines such as China. The phenolic compounds and polysaccharides present in these medicinal plants have been found to possess many properties such as anti-tumor, anti-oxidant, anti-inflammatory and anti-cancer effects on certain diseases. They can also be used for novel drug development and therapeutic purposes as well. Many diseases including skin diseases, heart diseases and cancer are caused by oxidative stress with imbalance of ROS resulting in damage to cell. Therefore, cells need anti-oxidant therapy in order to sustain the homeostasis of cells. ROS not only include free radicals but also non free radicals as well such as hydrogen peroxide, nitrogen reactive species and superoxide radicals [27]. All of these can be produced from toxins present inside the cells or outside in the environment such as consumption of alcohol and cigarettes, UV light and environmental pollution containing carcinogenic radiations [28].

2.3.1 PKB pathway

The naturally existing anti-oxidant system present in cells comprises two enzymatic and non enzymatic systems which function accordingly in response to high production of ROS species. Vitamin C and E belong to non

enzymatic system while others such as SOD, CAT and GR belong to enzymatic system. All of these anti oxidants are involved in providing the defense to cells from ROS. But when these ROS are produced in high amount, some medicinal compounds are needed to help these in preventing the onset of many diseases. These ROS are produced by mitochondria as an end product of ETC. The PI3K or PKB signaling pathways result in the formation of cancerous cells and tumors activated in consequences of ROS production. These pathways lead to an increase in cellular proliferation and cause a reduction in apoptosis of cells leading to cancer formation. The PI3K pathway is inhibited by PTEN (phosphates tensin homolog), whose level is found to be reduced by high production of ROS by mitochondria [29].

2.3.2 P53 pathway

The cancerous cells need an environment suitable for their growth which also includes the requirement of hypoxia induced factors such as HIFα. The genes required for the transcription of these factors are also activated by ROS production. The increased expression of these hypoxic genes also leads to the formation of cancer cells by increasing the level of angiogenesis in the cells. The production of ROS also causes the activation of some oncogenes such as c-Myc and Akt preferentially important for cancerous cells. The production of ROS can be inhibited by the tumor suppressor genes such as P53 leading to reduction in cancerous cell proliferation. Different types of cytokine receptors can also produce reactive oxygen species which weakens the immune system of the body as required for the cancer cells metastasis [30].

2.3.3 MMPs pathway

Recent studies show that liver cancer can also be caused due to production of hydrogen peroxide. The imbalance in cell cycle can also lead to cancer, caused by ROS production. The role of MMPs holds much importance as they can also aid in progression of cancer, with their increased expression caused by ROS [31]. The plant polysaccharides contain many compounds such as uronic acid which is an important component for the treatment of cancer related diseases [32]. Many plants contain polysaccharides that can be used for therapeutic purposes include jujube plant, tea plant, aloevera plant, apple, pumpkin, mulberry and wolf berry plants. The polysaccharides present in jujube plant also possess hepato protective properties against liver injury induced by CCl_4 by the enhanced expression of cas-3 and cas-9 (caspases).

2.3.4 STAT pathway

The experiments conducted on dietary *Z.jujube* showed that the polyphenols, flavonoids and terpenoids present in jujube plant decreased the expression of NF-κB, IL-6 and JAK-STAT pathway in mice with colon cancer caused due to dextran sodium sulfate. All of these pathways are involved in the formation of tumors and progression of cancer such as colon and colorectal cancer. The metastasis of cancer was inhibited in this study with the induction of apoptosis by the nutritional components of jujube plant. The reduction in tumorigenesis was examined by the expression of Bcl-2 and Bax apoptotic proteins. The expressions of all these mechanism and proteins were observed in cancer caused mice and treated mice and the difference showed the results by the comparing the both treated and untreated groups [33].

2.3.5 NF-κB pathway

Ursolic acid, the most important nutritional component of triterpenoids, is not only found in jujube plant but also many other plants. This compound was first discovered in apple waxes. It holds much importance as medicinal plant because of its characteristics such as anti-diabetic, anti-inflammatory, anti-oxidant and anti-cancer [34]. This compound also found to regulate different signaling pathways regarding cancer such as p53 pathway, Wnt pathway, KF-κB pathway and STAT3 pathway. The compound also regulates certain enzymes, transcription factors, growth factors and cytokines that affect the cancerous cells by regulating their proliferation, progression, angiogenesis and apoptosis. This compound also possesses some disadvantages such as poor availability, due to which it has been structurally modified by replacing the functional groups at specific carbon sites such as C-2, C-1 and C-28, to be used for drug development and to increase its pharmaceutical properties. Recent studies show that this compound can suppress cancer metastasis by arresting the cells in G1 phase of cell cycle by inhibiting the above mentioned pathways. Recent studies reported the efficient effects of the structural modification with enhanced anti-cancer potentials on different cell lines such HeLa (cervical cancer), HepG2 (hepatocellular carcinoma) and A549 (lung cancer) [24].

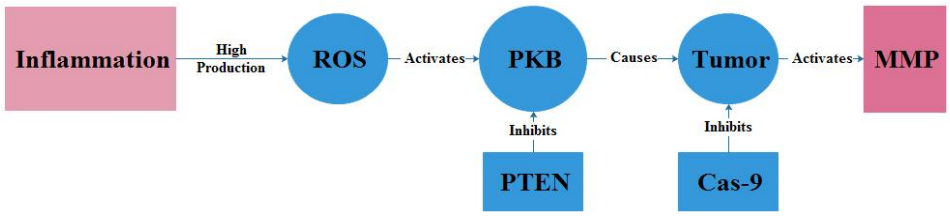

Fig. 1. PKB pathway in which PTEN (phosphate tensin homolog) and Cas-9 inhibits the PKB/PI3K to inhibit the formation of tumor caused by high production of Reactive Oxygen Species (ROS) such as free radical

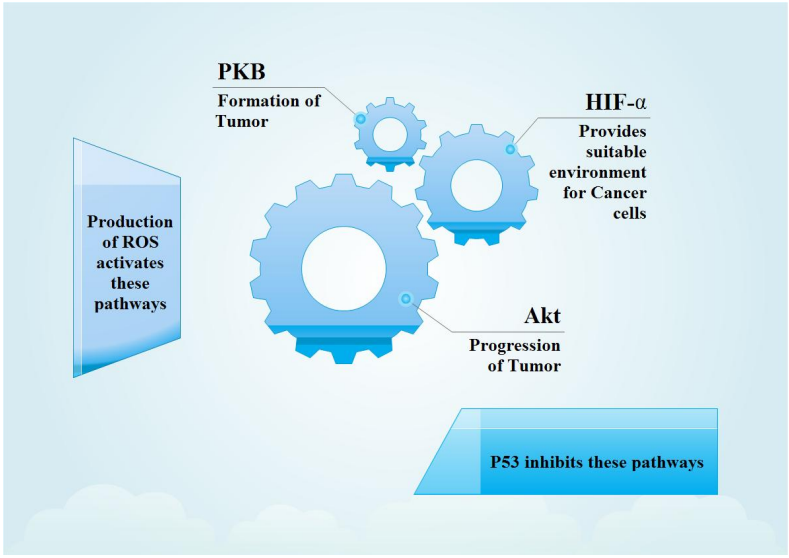

Fig. 2. p53 pathway inhibits the formation of tumor by PKB, HIF-α and Akt pathways to hinder these pathways in order to reduce the development and progression of tumor

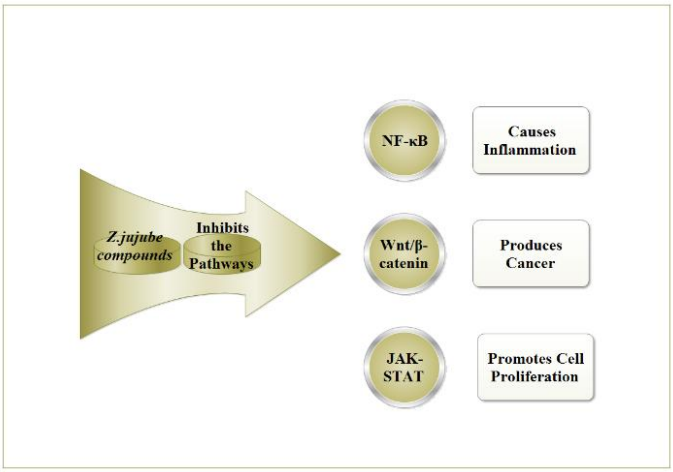

Fig. 3. NF-κB, Wnt and JAK-STAT pathways inhibited by the *Z. jujube* compounds to reduce the formation of tumor

The pathways including PKB, P53, Cas-9, NF-κB, JAK-STAT, Wnt and MMP are involved in regulating the anti-cancer potentials. Some of these pathways are involved in reducing cancer such as Cas-9 and P53 while others are involved in promoting the cancer. The mutation in others apart from these two causes the progression of tumor, increases proliferation and inhibits apoptosis. The anti-cancer potentials of polyphenolic compounds and triterpenoid acids show the regulation of these pathways either by inhibiting them or by activating them in order to promote or eradicate cancer cells.

3. ANTICANCER EFFECTS OF *Z. mauritiana*

This species of *Ziziphus* plant also belongs to Rhamnaceae family possessing anti-oxidant and anti-inflammatory properties. The tree of this species possess approximately 15 m height. The *Ziziphus* plant exists with 170 species with most of them using as medicinal plants [35]. The composition of ZM (*Z. mauritiana*) includes macronutrients and phytochemicals such as minerals, fatty acids, vitamins, amino acids, flavonoids, carotenoids and organic acids with anti-oxidant and anti-tumor activity [36]. The mostly widespread fruit of sub tropical and tropical regions is ZM. It is utilized in many different forms such as eaten either in dried form or candied and pickled form. The seed of this plant has been used to treat diseases since last century. Recent studies show that this specie of *Ziziphus* plant comprises aliphatic and phenolic compounds with flavonoids, steroids and most importantly the triterpenes. The triterpenes such as betulinic acid and ceanothic acid has also been shown to possess anti-tumor and anti-inflammatory with anti-oxidant properties [37]. The study depicts the importance of three particular zizimauritic acids (ZA) such as ZA-A, ZA-B and ZA-C and triterpenes such as betulinic acid, ceanothic acid and ceanothenic acid having anti-tumor activities against various cancer cell lines [38].The compounds present in plants extract also reported to possess anti-cancer, anti-diabetic, anti-oxidant and hypoglycemic properties [39]. The properties were studied with ethanolic fraction of plant extract [40]. The fruits of *Z.jujube* and *Z.mauririana* are produced by these two species of ziziphus plant and they are edible fruits. Three cultivated varieties of *Z.mauririana* including Gola ber, Sufi ber and Chambeli ber are commonly found and consumed in Pakistan especially in Sindh region. The extract of their fruits comprise many compounds but the flavonoids, phenolic acids and phytochemical holds medicinal importance. Phenolic acids are present in free form as well as in bound form or conjugated form. Different aqueous solvent mixtures are used to extract the free phenolics and various acids, bases or enzymes are used to extract the bound phenolics [41]. These phenolic compounds are extracted by aqueous fraction of dried plant materials. The bound or conjugated phenolic acids are present in the extract in combined form with sugars and additional bio-molecules. Different flovonoids have also been recognized present in these fruits. The fruits of this specie may possess oval or round shape with 2-6.5 cm length. The fruit resembles small apples being green in color and possess a sweetest taste. The anti-oxidants are present in rich amount in the fruits. The flaovonoids present in the plant have also been shown to possess cyto-toxicity properties. The nutrients and anti-oxidants present in diet have been shown to reduce the dietary problems caused by environmental agents. The fruits of ZM can be used in the manufacturing of pharmaceutical products as they hold much importance as nutraceutical components [41].

Recent studies show that anti-oxidant compounds have been reported to possess the anti-tumor, anti-inflammatory and anti-cancer activities in addition to different fractions of the plant extract such as ethanol, chloroform and methanol fractions [42]. The components of ZM plant include the phenolic compounds that are known to have great potential for anti-cancer properties and have been shown to be used widely in the treatment of certain types of cancer [43]. They have also been shown to possess unique structural features and biological properties making them excellent sources to treat cancer. The anti-oxidant components of ZM plant include the phenolics and flaovonoids compounds [41]. Recent studies report that chloroform fraction possessed higher concentration of phenolic compounds than methanol fraction whereas the concentration of falovonoids was reported to be high in methanol fraction in comparison to chloroform fraction. The anti-cancer activities of ZM plant extract have also been reported to decrease the progression of cancer cell lines by chloroform fraction of the extract [44]. The components of ZM plants include mostly palmitic acid and linoleic acid. The fruits of ZM comprise the nutritional composition including vitamin C, proteins and carbohydrates with some micro-nutrients such as calcium, potassium, sodium, phosphorus, copper, iron

and zinc [45]. The ZM constituents being most important include the terpenoids such as triterpenoids as acids e.g. oleanolic acid, betulinic acid and ursolic acid [46]. Other constituents include the flavonoids and polysaccharides.

3.1 Components

The importance of bioactive components of medicinal plants such as ZM extract has been known as ethno-medicine for more than 400 years as they have been used as traditionally medicine in China and other countries. One of the significant components of ZM is terpenoids which are chemicals released by plants possessing structures that contain oxygen as functional groups. They are also known as terpenes. They are secondary metabolites produced from specific plant species. They have been reported to present in the form of triterpenic acids in the extract of ZM [47]. The extract of the plant have been reported to possess anticancer activities against certain cell lines such as HeLa and HepG2 cell line and normal cell line (HGF) [48]. The anticancer property was investigated by the reduction in progression in cancer cells and induction of apoptosis in cell lines. The terpenoids also known to possess other properties as well such as anti-hyperglycemic, anti-diarrheal, anti-oxidant, anti-microbial and immuno-modulatory activities [49]. The terpenoids include many acids but important acids possessing pharmaceutical properties include the betulinic acid and oleanolic acid [45].

Another significant constituent of ZM extract is the betulinic acid. This triterpenic acid can be used for cancer treatment as it possesses the ability to induce apoptosis in cancer cells. It also possesses the ability to inhibit the enzymes. The betulinic acid has been known as anticancer compound by the NCI (National Cancer Institute). Its anti-tumor activity has been studied in many experiments by observing the reduction in tumor growth with an advantage of no side effects. Along with betulinic acid, its derivatives are also used to initiate apoptosis via mitochondrial pathway with caspases such as cas-3 and cas-9 [50]. The pharmaceutical properties and biological potency of betulinic acid and its derivatives have been enhanced by structural modifications such as the replacement of functional groups.

The other imperative compound is oleanolic acid (OA) and its derivatives. It is also triterpenic acid with hepato-protective and anti-tumor properties [51]. The hepato-protective activity of OA has been studies against liver damage caused by CCl_4. The anti-tumor activity of oleanolic acid was observed through the inhibition of growth of tumor mass and cancer cells in hepatocellular carcinoma (HepG2 cell line). The proliferation of cancerous cells was also inhibited. The effects of OA have been studies both in in-vitro and in vivo models. Not only the OA but its derivatives have also been found to possess anticancer activity against certain cancer cell line such as HeLa cells through the production of ROS and initiation of cell death. The other biological properties of OA have been studied such as anti-diabetic, anti-microbial, antihypertensive, anti-oxidant and anti-inflammatory [51].

The other significant compounds of ZM include the flavonoids and its derivatives. They have been known to possess anticancer, anti-inflammation, antioxidant, antiviral and hepato-protective properties in addition to pharmaceutical characteristics. The hepato-protective activity of flavonoids have been studied against liver damage caused by CCl_4 leading to reduction in levels of proteins such as albumin, ALT (alanine aminotransferase) and AST (aspartate aminotransferase) [44]. The anticancer activity has also been studied against HepG2 cell lines by the reduction in growth of cancer cells. The flavonoids found to possess the ability to reduce the production of ROS [52].

3.2 Mechanism

Liver cancer is also caused by the high production of ROS. The effects of ROS are reduced by the anti-oxidant enzymes. The phenolic compounds present in the plant extract are responsible for decreasing the level of proteins which increases in result of liver damage caused by ROS and causing the increase in levels of anti-oxidant enzymes. Recent studies depict the hepato-protective property with ethanol fraction against liver damage induced by CCl_4. The free radicals produced as result of ROS were transformed into beneficial products by the phenolic compounds and triterpenes present in the plant extract. The activity of anti-oxidant enzymes such as CAT, SOD and GSH was increased by the treatment of plant extract in liver cells. Oxidative stress results from the imbalance in the production of ROS and by escaping out from protective mechanisms. These conditions can lead to onset of cancer and other diseases such as heart diseases and diabetes.

The increased production of ROS can also result from the condition of hyperglycemia as in diabetes. These conditions also lead to the activation of pathways responsible for high production of ROS resulting in the low insulin secretion. The ROS defense mechanisms also exist in the cells whose capacity of neutralizing the ROS production can be increased by anti-oxidants present in plants extract such as SOD, CAT and GSH.[39]. The secondary metabolites produced from the plants such as phenolic acids are more important than flavonoids as they are involved in the regulation of anti-oxidant effects. The phenolic compounds present in plants are mostly anti-oxidant in nature. Recent studies reported that ZM has the ability to prevent the tumor growth by several mechanisms such as NF-κB, COX-2, MMP-9, TNF, cytokines such as chemokines, decreased level of growth receptors e.g. EGFR and inhibition of several protein kinases. The inhibition and prevention of tumor growth in cancer cells is followed by several mechanisms such as activating tumor associated macrophages and inducing apoptosis in cancer cells.

3.2.1 Betulinic acid mechanism

The mechanism of anti-tumor activity of betulinic acid involves the induction of apoptosis through mitochondrial pathway including caspases and anti-apoptotic Bcl family proteins such as Bcl-2 and Bax proteins to reduce the progression of cancer cells. This pathway is activated as the result of cancer therapies causing DNA damage and initiation of cellular stress conditions. The proteins are released from mitochondrial membranes (inner and outer) by their permeablization by betulinic acid. The process of permeablization is activated by the production of ROS. The expression of Bcl family protein is regulated by betulinic acid to cause the cell death in cancer cells. The cellular stress conditions are regulated by transcription factors such as NF-κB, regulated by betulinic acid. The mechanism is mediated by betulinic acid to result in apoptosis in cancer cell lines. The enzymes responsible for increased expression of angiogenesis are also inhibited by betulinic acid. The expression level of VEGF (vascular endothelial growth factor) is also regulated by betulinic acid as this factor is responsible for increased expression of angiogenic proteins leading to enhanced progression of cancer [53].

3.2.2 Oleanolic acid mechanism

The suggested mechanisms of anticancer activity of oleanolic acid (OA) include the enhanced expression of P53, COX-2 and induction of apoptosis via cell cycle arrest. As the P53 is tumor suppressor gene its mutation leads to onset of cancer by modifying into oncogenes. The COX-2 is tumor promoting gene. So both of them results into progression of cancer cells [54].The effect of OA reduces the expression level of COX-2 and increase the expression of normal P53 in order to induce apoptosis through cell cycle arrest at G_o phase. In addition to these pathways, another mechanism accounts that is the mitochondrial derived activation of caspases [55]. The other pathways also include the enhanced expression of tumor suppressor protein such as miRNA in order to treat the disease.

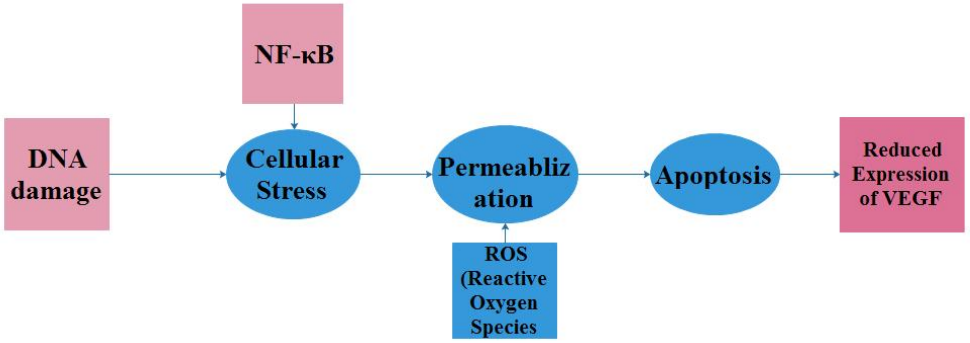

Fig. 4. Betulinic Acid mechanism in which DNA damage causes stress conditions leading to permeablization of mitochondrial membranes by ROS to initiate apoptosis and reduce the expression level of vascular endothelial growth factor (VEGF)

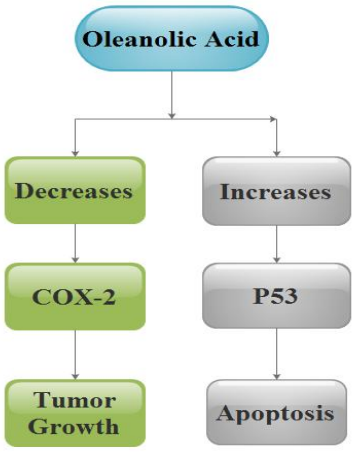

Fig. 5. Oleanolic Acid mechanism in which the OA increases the expression level of P53 to induce apoptosis and decreases the expression of COX-2 important for growth of tumor cells

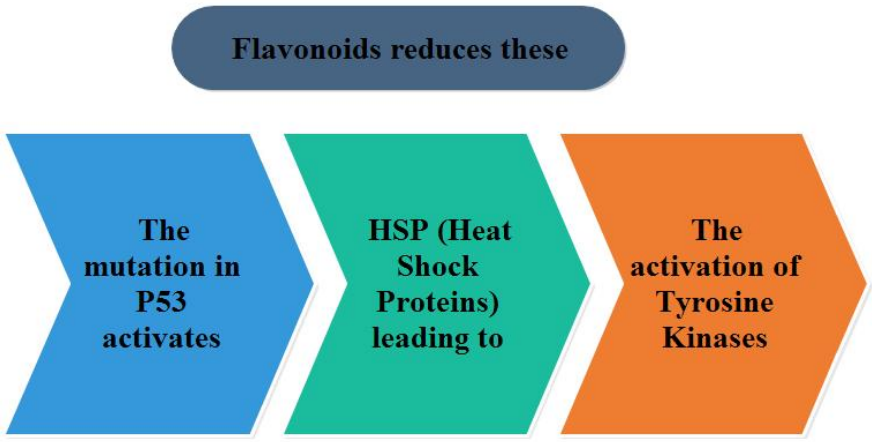

Fig. 6. The Flavonoids mechanism in which it inhibits the activation of mutated P53, heat shock proteins and tyrosine kinases to initiate apoptosis in cancer cells

3.2.3 Flavonoids mechanism

The mechanisms of flavonoids include the increased expression of P53 protein, the checkpoints in cell cycle, the inhibition of tyrosine kinases and proteins and reduction in the expression level of Ras protein. Recent studies depicts that flavonoids possess the ability to decrease the level of mutated P53 protein necessary for developing cancer. Some proteins are found to be associated with mutated P53 such as HSP (heat shock proteins) to stimulate the proliferation in cancer cells. The HSPs causes the mutation in checkpoints of cell cycle to surpass the cancer cells from cell cycle arrest. The activities of mutated P53, HSPs and the activation of tyrosine kinases responsible for tumor growth have been found to be inhibited by the pharmacokinetic effects of flavonoids.

4. CONCLUSION

The use of medicinal plants has been known for centuries to treat infectious diseases. These have been used as traditional medicine in China for more than 4000 years and other countries as well such as Asian countries (mostly Pakistan and India). Mostly the aerial parts of plants are used for the medicinal purposes. The Ziziphus plant has been focused importantly because of its pharmacological properties such as anti-inflammatory, antioxidant and anticancer

activities. Two different species of Ziziphus plant (*Z.jujube* and *Z.mauritiana* L.) have been discussed in this review: one is known as Chinese ber and the other is known as Indian ber. The nutritional composition of both species seems to be quite similar as in case of constituents but different in case of concentrations and mechanisms. The constituents of both species include the phenolic compounds, flavonoids and triterpenes such as triterpenic acids e.g. betulinic acid, oleanolic acid and ursolic acid. These compounds hold much importance as they regulate the mechanisms responsible for pharmacological characteristics.

The mechanisms of both species are somewhat different in the activation of cascades. The mechanisms of Ziziphus jujube include the PKB or PI3K, P53, MMPs, STAT and NF-κB mechanism. The mechanisms of other species which is Ziziphus mauritiana include the betulinic acid (BA), oleanolic acid (OA) and flavonoids mechanism. The major biological response for activating these mechanisms has been considered as the production of reactive oxygen species resulting from cellular stress conditions. These mechanisms have been studied in different cell lines such as HepG2 cell line and melanoma cells by means of plant extract with different fraction inclusive ethanol, chloroform and water. Some of these mechanisms have been studied in both in vitro and in vivo models. The effects of plants extract on the activation of these cascades depict the importance of this plant species as medicinal plants. It is concluded from this review that this plant can be used in the manufacturing of medicines for the treatment of infectious diseases such as cancer.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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