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Review Article
Pharmacological Aspects of Jujubes

Pierluigi Plastina

Department of Chemistry and Chemical Technologies, University of Calabria, 87036 Rende (CS), Italy

Abstract

Jujube has been traditionally used as folklore remedy for the treatment of many diseases. Several studies have addressed the question of the effectiveness of some of the putative pharmacological activities of jujube. In this study, the biological properties of jujube are discussed. The results from *in vitro* and animal studies are reported and, when possible, the occurrence of a single compound or a class of molecules responsible for these effects is highlighted. Jujube is a valuable fruit, from both nutritional and nutraceutical point of view, exerting a number of important pharmacological effects *in vitro* and *in vivo*. Results from human intervention studies are still required to allow a full validation of jujube health benefits.

Key words: *Ziziphus jujuba*, phenolics, triterpenic acids, jujube biological activities, traditional Chinese medicine

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Corresponding Author: Pierluigi Plastina, Department of Chemistry and Chemical Technologies, University of Calabria, 87036 Rende (CS), Italy

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jujube (Fig. 1), the fruit of *Ziziphus jujuba* Mill. (Rhamnaceae family) is widely distributed in many regions of Asia, Australia and the Mediterranean basin[1]. Jujube is also known as Chinese date, red date or tsao and it has been cultivated for more than 4000 years in China[2]. Jujube has been traditionally used fresh or processed (dried) as food, food additive and flavoring agent for thousands of years, due to its high nutritional values[3]. In addition, different parts of *Ziziphus jujuba* have been used (alone or as ingredient in more complex formulations) in Traditional Chinese Medicine (TCM) for calming down the mind and facilitate sleep and for the treatment of gastrointestinal problems, weakness, hepatitis, urinary troubles, skin infections, fever, pharyngitis, bronchitis, anemia and cancer[4-9]. Several studies have indeed confirmed that jujube displays many important biological properties, including antioxidant, neuroprotective, anticancer, anti-inflammatory, immunomodulatory, antiobesity, cardio, hepato and gastrointestinal protective activities, thus supporting the putative health benefits deriving from its consumption. This study deals with the pharmacological activities of jujubes. Results from *in vitro* and *in vivo* studies are discussed also and are summarized in Table 1 and 2, respectively. The chemical structures of the compounds responsible for jujube bioactivity are reported in Table 3.

IN VITRO STUDIES

Antioxidant and neuroprotective properties: Jujube extracts played a protective role in rat pheochromocytoma PC12 cells, a commonly employed model in the evaluation of neuroprotective effects, against tert-butyl hydroperoxide (tBHP)-induced cytotoxicity, stimulating the transcriptional factors of the antioxidant response element[10]. Water extracts containing high amounts of cyclic nucleotides (cGMP and cAMP) and polysaccharides showed the highest cell protecting effect against tBHP-induced cell death. Among water extracts from fruits at different ripening stages, green jujubes containing a significantly higher amount of flavonoids [namely (-)-catechin, (-)-epicatechin, procyanidyn B2 and quercetin 3-O-rutinoside] showed superior cell protective effects than ripe fruits[10]. The brain benefits of jujube were further studied by investigating neuronal differentiation of PC12 cells[11]. Neurite outgrowth of PC12 cells was found to be induced after administration of a chemically standardized jujube water extract containing nucleobases (at least 80 μg g^{-1} of dried extract), cGMP and cAMP (at least 150 μg g^{-1}) and flavonoids (quercetin 3-O-rutinoside, quercetin 3-O-galactoside, quercetin 3-O-β-D-glucoside and kaempferol 3-O-rutinoside, in a total amount of at least 35 μg g^{-1}). In addition, the expressions of neurofilaments (NFs), neuronal cell-specific cytoskeleton proteins, showed a concentration-dependent increase in jujube-treated cells, with inductions by ~150% for NF68 and NF160. Jujube-induced neurite outgrowth and neurofilament expression were inhibited by pre-treatment with H89, a cyclic AMP-dependent and selective protein kinase A inhibitor. Moreover, the phosphorylation of cAMP responsive element binding protein on PC12 cells was induced by jujube extract and blocked by H89. The cAMP contained in jujube water extract was found to be a crucial ingredient in neuronal differentiation of PC12. However, despite its relatively high amount, jujube cAMP did not fully account for the displayed jujube-induced beneficial effects. The researchers speculated that other compounds, such as flavonoids could be partly responsible for the effects of jujube. Chen *et al.*[11] investigated the expression of neurotrophic factors and antioxidant enzymes in cultured astrocytes treated with the aforementioned chemically standardized jujube water extract. Administration of jujube extract for 24 h significantly up-regulate mRNA expression of nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in a concentration-dependent manner. Moreover, treatment with jujube water extract induced mRNA expressions of several important antioxidant enzymes in a concentration-dependent manner[12]. Zare-Zardini *et al.*[13] investigated the effect of a new peptide (Snakin-Z)

Fig. 1: Fruit of *Ziziphus jujuba* Mill. (jujube)

Table 1: Overview of the biological activities of jujubes reported from *in vitro* studies

Biological activities	Cell line/enzyme	Jujube sample	Main findings	References
Antioxidant and neuroprotective activities	Rat pheochromocytoma PC12 cells	Dried jujube (from different regions of China and at different ripening stages) powder extracted twice in boiling water (1:20, w/v) for 1 h. The extracts were dried under vacuum	Jujube extracts protected PC12 cells against tBHP-induced cytotoxicity and stimulated the transcriptional activity of antioxidant response element. The antioxidant effects varied among jujube from different geographical origin and with ripening stages	Chen *et al.*[10]
	Rat pheochromocytoma PC12 cells	Dried jujube powder extracted twice in boiling water (1:20, w/v) for 1 h. The extracts were dried under vacuum	Jujube extract induced neurite outgrowth and concentration-dependent increase of neurofilaments (NF) expression. It also induced the phosphorylation of cAMP responsive element binding protein on PC12 cells All these effects were inhibited by pre-treatment with H89, a cyclic AMP-dependent and selective protein kinase A inhibitor	Chen *et al.*[11]
	Primary cultured rat astrocytes	Dried jujube powder extracted twice in boiling water (1:20, w/v) for 1 h. The extracts were dried under vacuum	Jujube water extract stimulated expressions of neurotrophic factors and anti-oxidant enzymes in cultured astrocytes. Pre-treatment with H89 attenuated the jujube-induced expression of neurotrophic factors	Chen *et al.*[12]
	Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes	Proteins of jujube water extract were suspended in solution as colloidal particles. After recentrifugation and dissolution in phosphate buffer, protein extract was passed through an ultramembrane with a 10 kDa cutoff. The filtrated solution was concentrated using a 1 kDa ultramembrane and lyophilized	A new peptide (Snakin-Z) showed inhibitory activity against AChE and BChE enzymes (IC_{50} values of 580 and 720 μg mL^{-1}, respectively) Snakin-Z had also DPPH radical scavenging activity (IC_{50} = 750 μg mL^{-1})	Zare-Zardini *et al.*[13]
Anticancer activities	K562, B16(F-10), SK-MEL-2, PC-3, LOX-IMVI and A549 tumor cell lines	Triterpenic acids isolated from jujube	Lupane-type triterpenic acids bearing a p-coumaroyl moiety at the C-3 position showed a higher cytotoxicity than non-esterified triterpenic acids	Lee *et al.*[17]
	Human hepatoma cells (HepG2)	Chloroform fraction of the initial jujube crude extract	The extract concentration-dependent effect on apoptosis, G1 arrest at a low concentration (100 μg mL^{-1}) and G2/M arrest at a higher concentration (200 μg mL^{-1})	Huang *et al.*[14]
	HeLa (human cervical carcinoma cell line), HEp-2 (human larynx carcinoma cell line) and Jurkat (T cell leukemia)	Water extract of dried jujube fruit at different concentrations (0-1 mg mL^{-1})	Induction of apoptosis on human tumor cell lines, HEp-2, HeLa and Jurkat cell lines; jurkat leukemic line was found the most sensitive with IC_{50} of 0.1 μg mL^{-1}	Vahedi *et al.*[15]
	Estrogen receptor alpha (ERα) positive MCF-7 and ERα negative SKBR3 human breast cancer cells	Lyophilized fruits extracted with various solvents	Two fractions containing triterpenic acids exerted significant antiproliferative effects in MCF-7 (IC_{50} values of 7.64 and 1.69 μg mL^{-1}) and SKBR3 (IC_{50} values of 6.21 and 3.70 μg mL^{-1}). Apoptotic cell death was induced in both malignant breast cells	Plastina *et al.*[16]
	Melanoma cells	Deproteinized polysaccharide (DPP) fractions were obtained through boiling water extraction, follwed by ethanol precipitation, deproteinization and dialysis	DPP inhibited the proliferation of melanoma cells in a concentration-dependent manner. The IC_{50} of 3990 and 3360 μg mL^{-1} after 24 or 48 h of 76 treatment, respectively DPP induced arrest at the G2/M phase, leading to the formation of an apoptotic body and to an increase of the activity of caspase-3 and caspase-9	Hung *et al.*[20]
	HeLa cervical, A549 lung and U937 lymphoma cell lines	Extraction with 80% methanol/water of jujube from eight different growth stages (S1-8), followed by centrifugation	All cancer cells were concentration-dependently inhibited by extracts at the earlier stage of growth (S1). The S1-S8 inhibited HeLa cells, while inhibition progressively decreased with maturation for Hel299 and A549	Choi *et al.*[22]
	MCF-7 cells	Betulinic acid isolated from sour jujube fruits and complexed with β-cyclodextrin (β-CD)	The complex was found to inhibit the proliferation of cells in a concentration-dependent way, arresting cell cycle in the G2/M phase and inducing apoptosis via the mitochondria transduction pathway	Sun *et al.*[19]

Table 1: Continue

Biological activities	Cell line/enzyme	Jujube sample	Main findings	References
	HepG2 cells and MCF-7 cells	Triterpenic acids isolated from jujube	Some triterpenic acids strongly inhibited proliferation with IC_{50} values of 2.5 μg mL^{-1}	Qiao *et al.*[18]
	Cervical cancer cells (SIHA), colon cancer cells (HCT116), liver cancer cells (HepG2), breast cancer cells (MCF-7), skin cancer cells (SK-MEL2), leukemic T cells (Jurkat) and leukemic monocyte cells (U937)	Water extracts from six cultivars of jujubes at different ripening stages	Bombay variety was found to possess moderate cytotoxicity against the Jurkat cell line with IC_{50} values of 375 μg mL^{-1}, but apoptosis was not induced	Siriamornpun *et al.*[21]
Anti-inflammatory and Immunomodulatory activities	Rat peritoneal macrophages	Six fractions extracted from *Z. jujuba*	Triterpenic acids fraction concentration-dependently inhibited NO production in cells activated by Radix Kansui	Yu *et al.*[23]
	Spleen cells	Polysaccharide fraction extracted from jujube and further purified to give pectic Ju-B-2 and Ju-B-3	The Ju-B-2 induced a concentration-dependent proliferation of spleen cells	Zhao *et al.*[24]
	Splenocytes and peritoneal macrophages	Water-soluble crude polysaccharides from jujube were purified via DEAE-sepharose CL-6B column chromatography to give various fractions	The ZSP3c (a pectin-rich fraction) concentration-dependently stimulated the proliferation	Li *et al.*[25]
	RAW 264.7 macrophages	Dried jujube fruit powder extracted twice in boiling water (1:20, w/v) for 1 h. The extracts were dried under vacuum	Jujube water extract for 24 h stimulated the transcriptional expression of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in cultured RAW 264.7 macrophages. By contrast, pretreatment with jujube water extract suppressed the expression of IL-1β and IL-6, but not for TNF-α in LPS-stimulated macrophages	Chen *et al.*[5]
	Human hepatocellular carcinoma Hep3B cells	Dried jujube fruit powder extracted twice in boiling water (1:20, w/v) for 1 h. The extracts were dried under vacuum	Jujube water extract stimulated erythropoietin and hypoxia-inducible factor-1α mRNA expression in a concentration-dependent manner	Chen *et al.*[27]
Antiobesity activities	Mouse embryo 3T3-L1 preadipocytes	Jujube extract with different solvents (chloroform, ethyl acetate, n-butanol, and water)	Chloroform extract (CHCl3–F) efficiently suppresses lipid accumulation and glycerol-3-phosphate dehydrogenase activity	Kubota *et al.*[28]
Cardioprotective activities	Human peripheral mononuclear cells	Crude plant extracts; isolated triterpenoid compounds	Extracts from fruit and seed inhibited foam cell formation induced by acetylated LDL; oleanonic acid, pomolic acid and pomonic acid were the major active compounds	Fujiwara *et al.*[29]

Table 2: Overview of the biological activities of jujubes reported from *in vivo* studies

Biological activities	Model	Jujube sample	Main findings	References
Antioxidant and neuroprotective activity	Male gerbils (6 months-old)	The dried fruits of *Z. jujuba* (200 g) except seeds were extracted with methanol (3×3L) by maceration for 1 week at room temperature. The extracts were combined and concentrated in vacuo at 40°C to give a methanolic extract (ZJE)	Neuronal nuclei-immunoreactive neurons were abundant (58.4%) in the hippocampal CA1 region 4 days after I/R in the ZJE-treated ischemia group, compared to those in the vehicle-treated ischemia group (11.3%) Levels of hydroxynonenal were much lower in the ZJE-treated ischemia group than those in the vehicle-treated ischemia group	Yoo *et al.*[30]
	Male Wistar rats (150-200 g)	Jujube fruit powder was extracted using hot distilled aqueous and 50% methanol for 3 h (three times) The collected filtrates were concentrated under vacuum at 40°C. The semisolid form of the extract was dried in a vacuum tray drier to obtain the hydroalcoholic extract of *Ziziphus jujube* (HEZJ)	Pretreatment with HEZJ produced significant dose-dependent protection against PTZ-induced seizures and 66.7% protection against tonic hind limb extension in the MES seizure model Pretreatment with HEZJ reversed the significant decrease in cholinesterase activity observed in the PTZ and MES models	Pahuja *et al.*[31]
	Male Wistar rats (150-250 g)	Jujube fruit powder was extracted by distilled water and methanol (1:1 v/v). This extract was filtered and concentrated under reduced pressure on a rotary evaporator and then lyophilized to obtain *Ziziphus jujube* (ZJ) extract	In shuttle box test, ZJ extract (500 and 1,000 mg) significantly increased step-through latency in rat model of AD. The ZJ extract (500 and 1,000 mg kg^{-1} day^{-1}) increased plasma levels of antioxidants and decreased plasma levels of malondialdehyde compared to control	Rabiei *et al.*[32]
Anti-inflammatory and immunomodulatory activities	Wistar albino rats (180-240 g)	Dried coarse powder extracted with 60% ethanol to yield jujube extract (ZJ)	The ZJ significantly attenuated the effect of carrageenan in rat paw at 100, 200 and 400 mg kg^{-1}; ZJ inhibited granuloma formation at 200 and 400 mg kg^{-1} and markedly decreased serum nitrite/nitrate at 200 and 400 mg kg^{-1}	Goyal *et al.*[33]
	Three months-old Kunming mice (males and females, 20±2 g)	Water-soluble crude polysaccharides fractioned by DEAESepharose CL-6B anion-exchang e column (4.5×50 cm), Sepharose CL-6B (2.6×60 cm) polysaccharide fractions from *Z. jujuba* cv. Jinsixiaozao	The CZSP dramatically increased thymus and spleen indices and peritoneal macrophages CZSP, ZSP3, ZSP3c, ZSP4 and ZSP4b induced the proliferation of spleen lymphocyte	Li *et al.*[25]
Anti-allergic activity	Wistar rats (150-200 g), Swiss albino mice (20-25 g), Dunkin-Hartley guinea pigs (350-400 g) of either sex	The dried powder of fruits was extracted by cold maceration for 72 h with ethanol (95%). The ethanolic extract was filtered and concentrated in rotary vacuum evaporator	Treatment with extract of *Z. jujuba* at all doses significantly prevented the milk-induced eosinophilia, decreased passive cutaneous and active anaphylactic reactions. Jujube extract inhibited acetylcholine and histamine induced tracheal chain contraction and also antigen induced contraction of sensitized guinea pig ileum	Naik *et al.*[34]
Cardioprotective activity	Nine weeks-old male Wistar rats (200-230 g)	Jujube peel exhaustively extracted to give jujube peel free phenol (JPFP) and jujube peel bond pheno (JPBP)	Pre-administration of JPFP or JPBP can prevent ISO-induced myocardial l ischemia by inhibiting increases in levels of MDA and activities of Ca^{2+}-ATPase and Mg^{2+}-ATPase, reductions in activities of superoxide dismutase, glutathione peroxidase, creatine kinase, lactate dehydrogenase and Na^{+}/K^{+}-ATPase in the rats induced by ISO	Cheng *et al.*[35]
	Thirty two male 2 months old Wistar rats	Undescribed jujube extract	Jujube extract could reduce heart lipid peroxidation level and Bax expression, increase heart antioxidant enzymes activities and Bcl-2 expression and improve heart function	Liang and Juan[36]

Table 2: Continue

Biological activities	Model	Jujube sample	Main findings	References
Gastrointestinal protective activity	32 male golden syrian hamsters (6 weeks old)	Water-soluble carbohydrate concentrate (WSCC) obtained from dried jujube distilled with boiled water for 2 h, then filtered and dried by lyophilization	The WSCC (5.0 and 15 g kg^{-1} diet) shortened gastrointestinal transit time (34.2-57.3%), reduced cecal ammonia (58.1-60.3%), elevated total short-chain fatty acid concentrations in cecum (3-4 fold), increased fecal moisture (147-170%), reduced daily fecal ammonia output (1.9-75.8%) and decreased the activities of β-D-glucuronidase (73.0-73.8%), β-D-glucosidase (58.2-85.7%), mucinase (46.2-72.6%) and urease (31.9-48.7%) in feces	Huang *et al.*[37]
	Rabbits	Jujube powder (200 g) extracted three times with boiled water (1500 mL) for 5 h each time; the concentrated extract precipitated with 70% ethanol for 12 h, the precipitates lyophilized to obtain polysaccharides	Jujube extract reduced intestine MDA level and increased antioxidant enzyme activities in rabbits with I/R of the small intestine	Wang[38]
Hepatoprotective activity	Male ICR mice	Jujube ethanolic extract (FZJ)	Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found significantly lower following the administration of FZJ at the dose of 200 mg kg^{-1}. The FZJ administration inhibited lipid peroxidation and increased the activities of endogenous antioxidant enzymes	Shen *et al.*[8]
	Kunming male mice (18-22 g)	Dried jujube fruit powder soaked in water (1:20, w/v) at 80°C for 160 min. After three cycles, solutions filtered and concentrated, then precipitated with 95% ethanol at 4°C for 24 h, refined pellets dialyzed with water for 4 days (cutoff Mw 0.8×104 Da); retentate deproteinized by freeze–thaw process repeated 10 times followed by filtration; supernatant was lyophilized	Administration of polysaccharides derived from *Zizyphus jujube* reduced activities of CCl_4-elevated ALT, AST and LDH in serum and hepatic MDA level at 400 mg kg^{-1}; better profile of HI, normal GSH-Px and SOD activities in liver	Wang *et al.*[39]
	Kunming male mice (17-23 g)	Dried jujube fruit powder soaked in water (1:20, w/v) at 80°C for 160 min. After three cycles, solutions filtered and concentrated, then precipitated with 95% ethanol at 4 °C for 24 h, refined pellets dialyzed with water for 4 days (cutoff Mw 0.8×104 Da); retentate deproteinized by freeze–thaw process repeated 10 times followed by filtration; supernatant was lyophilized	Administration of polysaccharides derived from *Zizyphus jujube* at a dose of 400 mg kg^{-1} b.wt., significantly reduced the serum levels of glucose, insulin, TC, TG, LDL-C and VLDL-C. The ZSP also markedly improved the HDL-C level, homeostasis model assessment for insulin resistance (HOMA-IR) and b-cell function (HOMA-b) and decreased the atherogenic index (AI) of fructose-treated mice	Zhao *et al.*[40]

Table 3: Chemical structure of the compounds responsible for jujube bioactivity

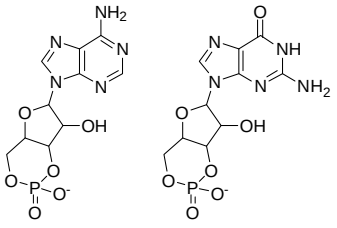
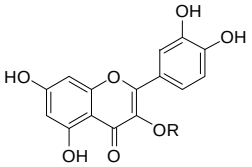
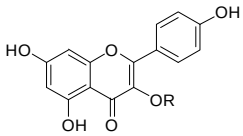
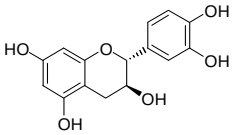
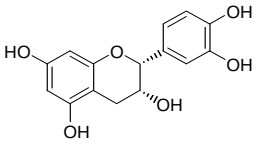
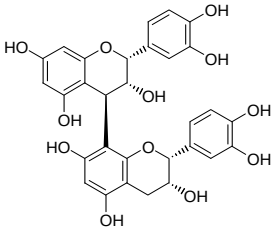
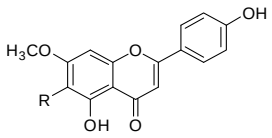
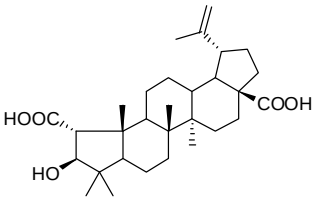
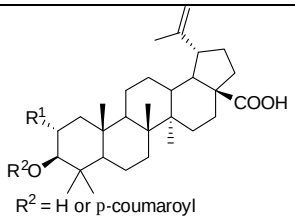
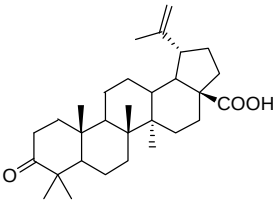
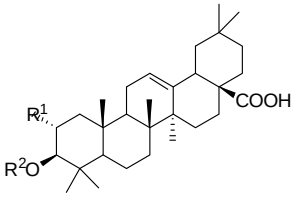
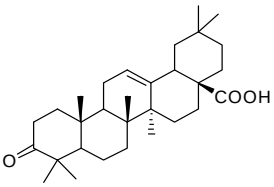
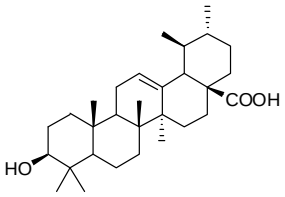
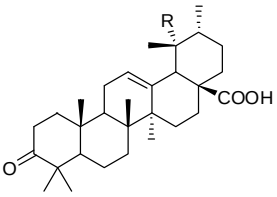
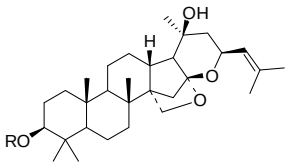
Main class	Name	Structure	References
Nucleotides	Cyclic nucleotides	 cAMP cGMP	Chen *et al.*[5,10,11,27]
Flavonoids	Quercetin glycosides	 R = Sugar moiety	Chen *et al.*[5,10,11,27] and Choi *et al.*[22]
	Kaempferol glycosides	 R = Sugar moiety	Chen *et al.*[11,27]
	Catechin		Chen *et al.*[10]
	Epicatechin		Chen *et al.*[10]
	Procyanidin B2		Chen *et al.*[10]
	Spinosin	 R = Sugar moiety	Pahuja *et al.*[31]
Triterpenes	Ceanothic acid (R^1 = αCOOH and R^2 = βOH) Epiceanothic acid (R^1 = βCOOH and R^2 = βOH)		Plastina *et al.*[16] Plastina *et al.*[16]

Table 3: Continue

Main class	Name	Structure	References
	Alphitolic acid (R^1 = OH) Betulinic acid (R^1 = H)	 R^2 = H or p-coumaroyl	Plastina *et al.*[16] and Lee *et al.*[17] Plastina *et al.*[16], Lee *et al.*[17], Sun *et al.*[19] and Naik *et al.*[34]
	Betulonic acid		Plastina *et al.*[16]
	Oleanolic acid (R^1 = H) Maslinic acid (R^1 = OH)	 R^2 = H or p-coumaroyl	Plastina *et al.*[16], Lee *et al.*[26], Fujiwara *et al.*[29] and Naik *et al.*[34] Plastina *et al.*[16] and Lee *et al.*[26]
	Oleanonic acid		Plastina *et al.*[16]
	Ursolic acid		Plastina *et al.*[16]
	Ursonic acid (R = H) Pomonic acid (R = OH)		Plastina *et al.*[16] Plastina *et al.*[16] and Fujiwara *et al.*[29]
	Jujubosides	 R = Sugar moiety	Pahuja *et al.*[31]

from jujube in inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChe) enzyme activity. The AChE and BChE were significantly inhibited by Snakin-Z with IC_{50} values of 580 and 720 μg mL^{-1}, respectively.

Anticancer properties: Huang *et al.*[14] investigated the anticancer activity of jujube in human hepatoma cells (HepG2) and found that jujube extracts decreased the viability of the cells. Chloroform fraction of the initial crude extract was the most effective, inducing a concentration-dependent effect on apoptosis, G1 arrest at a low concentration (100 μg mL^{-1}) and G2/M arrest at a higher concentration (200 μg mL^{-1}). The induction of apoptosis has been reported to be the mechanism through which jujube extracts exert anticancer activities in different human tumor cell lines, including HEp-2, HeLa and Jurkat cell lines[15]. The inhibitory effects of jujube extracts on breast cancer have been also investigated[16]. Fractions ZE2 and ZE4, containing triterpenic acids, exerted the highest antiproliferative effects on estrogen receptor alpha (ERα) positive MCF-7 (IC_{50} values of 7.64 and 1.69 μg mL^{-1}, respectively) and ERα negative SKBR3 (IC_{50} values of 6.21 and 3.70 μg mL^{-1}, respectively) human breast cancer cells. Moreover, ZE2 and ZE4 induced apoptosis in both cell lines and did not affect cell viability of both normal human fibroblasts BJ1-hTERT and nonmalignant breast epithelial MCF-10A cells[16]. The key role of some triterpenic acids present in jujube in inducing cytotoxicity in several tumor cell lines was already known. Lupane-type triterpenic acids bearing a p-coumaroyl moiety at the C-3 position showed a higher cytotoxicity than non-esterified triterpenic acids, suggesting that the esterification of the alphitolic acids with a p-coumaroyl group could be a key structural feature in inducing cytotoxic activity[17]. More recently, the proliferation of HepG2 and MCF-7 cells were strongly inhibited following the administration of triterpenic acids isolated from jujube, with IC_{50} values lower than 2.5 μg mL^{-1} [18]. Betulinic acid isolated from sour jujube fruits was complexed with β-cyclodextrin (β-CD) to improve its solubility and study its anti-proliferative and apoptotic effects on MCF-7 cells[19]. The complex was found to inhibit the proliferation of cells in a concentration-dependent way, arresting cell cycle in the G2/M phase and inducing apoptosis via the mitochondria transduction pathway. In particular, the complex was found to significantly inhibiting B-cell lymphoma 2 (Bcl-2) expression and promoting Bcl-2-like protein 4 (Bax) expression, causing caspase-3 and caspase-9 cascade activation[19]. Two polysaccharide fractions isolated from jujube were found to inhibit the proliferation of melanoma cells in a concentration-dependent manner and to induce arrest at the G2/M phase, leading to the formation of an apoptotic body and to an increase of the activity[20] of caspase-3 and caspase-9. Water extracts from several jujube varieties were investigated for their anticancer properties and one of them (Bombay) was found to possess moderate cytotoxicity against the Jurkat cell line with IC_{50} values of 375 μg mL^{-1}. However, it did not significantly induce cell death via apoptosis[21]. Jujube extracts from eight different growth stages (S1-8) inhibited the growth of HeLa cervical cancer cells in a concentration-dependent manner, while only the extracts from the early stages inhibited Hel299 normal lung and A549 lung cancer cell growth. The researchers correlated these latter findings with the content of certain flavonoids (quercetin 3-rutinoside and quercetin 3-robinobioside, mainly) and antioxidant activity that were found to decrease as the fruit matured[22].

Anti-inflammatory and immunomodulatory properties: Jujube is one of the ingredients of a Chinese formulation (Shi Zao) traditionally used to alleviate the severe inflammatory and irritant effects of *Euphorbia* plants. Yu *et al.*[23] provided a rationale for these anti-inflammatory properties, reporting that jujube extracts containing triterpenic acids strongly decreased the levels of Nitric Oxide (NO) and tumor necrosis factor (TNF)-α in rat peritoneal macrophages stimulated with *Euphorbia kansui* or prostratin, a phorbol ester isolated from *Euphorbia fischeriana*. Several studies report that jujube has immune stimulatory activity, mainly due to its polysaccharide content. Pectin (Ju-B-2) extracted from jujube and consisting of a polygalacturonan backbone with rhamnogalacturonan interspersed in the main chain induced a concentration-dependent proliferation of spleen cells. By contrary, a simple polygalacturonan (Ju-B-3) was inactive. The presence of rhamnogalacturonan and the side chains were suggested to be key factors in stimulating cell proliferation[24]. Polysaccharide fractions extracted from *Z. jujuba* cv. Jinsixiaozao (ZSP) were found to stimulate the proliferation of splenocytes and peritoneal macrophages[25]. Interestingly, pectin with a degree of esterification of 49% was abundant in the most active fraction (ZSP3c). Jujube extracts are also known to have anti-complementary activities. Some triterpenic acids belonging to the oleanane-type family exhibited significant anticomplementary activities with IC_{50} values ranging from 62.4-88.9 μg mL^{-1}. Interestingly, other triterpenes were inactive, suggesting that the oleanane pattern is a crucial structural feature in inhibiting the hemolytic activity of human serum against erythrocytes[26]. Jujube water extracts containing nucleobases, cyclic nucleosides and flavonoids[11] have been shown to exert dual immune-modulating properties by regulating the expressions of pro-inflammatory cytokines in macrophages. Under normal conditions, mRNA expression of interleukin (IL)-1β, IL-6 and TNF-α were up-regulated after 24 h treatment with jujube extract in cultured RAW 264.7 macrophages. By contrast,

the over-expression of IL-1β and IL-6, in lipopolysaccharide (LPS)-stimulated macrophages was abolished upon administration of the jujube water extract, at both mRNA and protein levels[5]. Jujube effect in stimulating hematopoietic function has been investigated, by evaluating the expression of erythropoietin in cultured Hep3B human hepatocellular carcinoma cells. Administration of the aforementioned jujube water extract[11] stimulated erythropoietin expression in a concentration-dependent manner and up to ~100% of increase. Moreover, jujube water extract induced the transcriptional activity of the regulator for erythropoietin expression in Hep3B cells transfected with a plasmid containing hypoxia response element[27].

Antiobesity properties: Jujube extracts have been shown to circumvent adipogenesis in 3T3-L1 preadiocytes. Lipid accumulation and glycerol-3-phosphate dehydrogenase activity were suppressed following administration of jujube extracts, while cell viability was unaffected. The major inhibitory effects were obtained in the case of the chloroform fraction, which was able to down-regulate the expression of key adipogenic transcription factors, including peroxisome proliferator-activated receptor (PPAR)-gamma and CCAAT enhancer binding proteins (C/EBPs)[28].

Cardioprotective properties: Fujiwara *et al*.[29] reported that triterpenes from jujube were able to inhibit foam cell formation induced by acetylated LDL in human macrophages. Among the compounds tested, triterpenic acids, including oleanonic, pomolic and pomonic acids were found as the most active; suggesting that the carboxylic group at C-28 is an important structural feature in inhibiting foam cell formation.

IN VIVO STUDIES

Antioxidant and neuroprotective properties: The neuroprotective properties of jujube extract (ZJE) were investigated in gerbils under conditions of ischemic damage. Neuronal nuclei-immunoreactive neurons were more abundant in the hippocampus of the ZJE-treated ischemia group than in the vehicle-treated ischemia group 4 days after ischemia/reperfusion (I/R). Superoxide dismutase (SOD) and brain-derived neurotrophic factor were higher in the ZJE-treated ischemia group than in the vehicle-treated ischemia group 4 days after I/R. In addition, the amount of hydroxynonenal, a marker of lipid peroxidation, was significantly lower in the ZJE-treated ischemia group than in the vehicle-treated ischemia group after I/R[30]. Pahuja *et al*.[31] reported on the anti-epileptic properties of jujube extracts in rats. Administration of hydroalcoholic extract of jujube (HEZJ, containing spinosin and jujubosides as the major components) resulted in up to 100% protection against experimental seizure models of epilepsy in rats challenged with pentylenetetrazole (PTZ) or maximal electroshock (MES). In addition, the PTZ (or MES)-induced oxidative stress and decrease in cholinesterase activity were significantly reversed by HEZJ, leading to significant improvements in memory and learning. The memory dysfunction in Alzheimer's disease has been associated with cortical cholinergic deficiency and loss of cholinergic neurons of the nucleus basalis of Meynert (NBM). A standardized jujube hydroalcoholic extract administration had repairing effects on memory and behavioral disorders produced by NBM lesions in rats, by activating choline acetyltransferase and may have beneficial effects in treatment of AD patients[32]. Total Phenolic Content (TPC) of the extract was 48.8 mg of gallic acid equivalent (GAE) per gram dried extract. Total flavonoid and flavonol contents were 9.1 and 8.0 mg of rutin equivalent (RE) per gram dried extract, respectively.

Anti-inflammatory and immunomodulatory properties: An hydroalcoholic extract of jujube (ZJ) was found to exert anti-inflammatory properties in both acute and chronic models of Inflammation in rats. The ZJ induced a dose-dependent reduction of edema induced by sub-plantar administration of carrageenan in rat hind paw. Moreover, ZJ significantly decreased the formation of granuloma tissue and nitrite/nitrate level induced by interscapular implantation of a sterile cotton pellet. The ZJ was found to contain jujubosides, flavonoids and terpenes but the bioactive compounds responsible for the reported effects were not investigated[33]. The polysaccharide fractions from *Z. jujuba* cv. Jinsixiaozao (ZSP) discussed earlier was also reported to enhance thymus and spleen indices in mice[25]. Also in this case, the most active fraction (ZSP3c) was rich in pectin with a degree of esterification of 49%.

Anti-allergic properties: Naik *et al*.[34] evaluated the anti-allergic and anti-anaphylactic activity of the ethanolic extract of jujube in asthma and allergy animal models. Milk-induced eosinophilia and degranulation of mesenteric mast cells were significantly prevented by jujube extract. In addition, jujube extract inhibited acetylcholine as well as histamine-induced tracheal chain contraction and antigen-induced contraction of sensitized guinea pig ileum. A preliminary phytochemical analysis of the extract showed the presence of triterpenoids (betulinic acid, oleanolic acid, betulin and lupeol), alkaloids, carbohydrates, proteins and flavonoids.

Cardioprotective properties: Cheng *et al.*[35] evaluated the protective effect of phenolics from Chinese jujube against Myocardial Injury (MI) in rats. Pre-administration of both free and bound phenolics from jujube peel (JPFP and JPBP, respectively) significantly inhibited the production of malondialdehyde (MDA) and the activities of Ca^{2+}-ATPase and Mg^{2+}-ATPase induced by isoproterenol (ISO) or aluminum chloride. In addition, the reduction of superoxide dismutase (SOD), glutathione peroxidase, creatine kinase, lactate dehydrogenase and Na^{+}/K^{+}-ATPase activities was circumvented upon treatment with JPFP or JPBP. Sun and Jiang investigated the effect of an undescribed jujube extract on oxidative injury in heart muscles of exhausted training rats. Their results showed that jujube extract was able to inhibit lipid peroxidation in heart and Bax expression, to increase the activity of antioxidant enzymes and Bcl-2 expression, thus improving heart functions[36].

Gastrointestinal protective properties: A few studies have been carried out to provide a rationale for the anecdotal use of Chinese jujubes for the treatment of gastrointestinal symptoms. The effect of Water Soluble Carbohydrate Concentrate (WSCC) extracted from jujube and consisting of glucose, fructose, pectin polysaccharide and hemicellulose was investigated on several intestinal and fecal indices in a hamster model. The WSCC was found to effectively reduce gastrointestinal transit time and the amount of daily fecal ammonia in hamsters, thus allowing intestinal health maintenance[37]. Jujube polysaccharides, containing xylose (31.3%), glucose (23%), fructose (21.6%) and mannose (12.9%) as main monosaccharide units were found to significantly reduce intestine malondialdehyde (MDA) level and increase the activity of antioxidant enzymes in a rabbit model stimulated with I/R of the small intestine[38].

Hepatoprotective properties: Jujubes are traditionally used for the treatment of hepatitis in Northern China. The hepatoprotective properties of jujube ethanolic extract (FZJ) on liver injury induced by carbon tetrachloride (CCl_4) were investigated in male ICR mice[8]. Two biomarkers of hepatic injury in blood, namely alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found significantly lower following the administration of FZJ at the dose of 200 mg kg^{-1}. Moreover, FZJ administration inhibited lipid peroxidation and increased the activities of endogenous antioxidant enzymes. Wang *et al.*[39] investigated the effects of jujube heteropolysaccharides (ZSP), containing L-arabinose as the main monosaccharide unit, on CCl_4-induced liver oxidative stress in mice. The activities of ALT, AST and lactic dehydrogenase in serum and the hepatic malondialdehyde level were significantly reduced following the administration of ZSP at a dose of 400 mg kg^{-1}. Moreover, ZSP-treated mice showed a better profile of hepatosomatic index and antioxidant system with normal activity of glutathione peroxidase and SOD in the liver. Zhao *et al.*[40] investigated the effects of jujube polysaccharides (ZSPs) on fructose-induced insulin resistance and dyslipidemia in mice. The serum levels of glucose, insulin and dyslipidemia increased by 20% fructose water were significantly reduced upon administration of ZSP at a dose of 400 mg kg^{-1}. Hepatoprotective effect of ZSP against liver steatosis induced by a high-fructose diet was also confirmed by histopathological tests. These findings indicate that jujube polysaccharides may ameliorate insulin resistance and dyslipidemia in fructose-treated mice.

CONCLUSION

This study shows that jujube exerts a number of important pharmacological activities and can be therefore considered a valuable source of nutraceuticals. However, some of the reported effects of jujube are still anecdotal and have not been fully correlated with the occurrence of one or more specific bioactive compounds. In addition, the biological properties of jujube have been observed only in studies using *in vitro* and/or animal models. Human intervention studies are therefore indispensable to obtain reliable scientific evidence of the health benefits of jujube, in order to validate it as a healthy fruit.

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