



THE CHEMICAL CONSTITUENTS AND PHARMACOLOGICAL EFFECTS OF *CALENDULA OFFICINALIS* - A REVIEW

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ABSTRACT

Herbal plants provide a rich source for health care to prevent and treat different pathological states. *Calendula officinalis* is an aromatic, erect, annual herb belong to the family asteraceae, it contained a wide range of chemical constituents including saponins, triterpenes, triterpendiol esters, flavonoids, steroids, tannin, quinines, coumarins, carotenoids, amino acids, polysaccharides, essential and volatile oils and many other chemical groups. *Calendula officinalis* exerted many therapeutic effects including antibacterial, antifungal, anthelmintic, antiviral, cytotoxic, antioxidant, anti-inflammatory, analgesic, hepatoprotective, cardioprotective, gastroprotective, wound healing and many other effects. The present review will highlight the chemical constituents and the pharmacological and therapeutic effects of *Calendula officinalis*.

Keywords: *Calendula officinalis*, Pharmacology, Constituents, Review.

INTRODUCTION

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, pesticides and food additives [1-46]. *Calendula officinalis* contained a wide range of chemical constituents including saponins, triterpenes, triterpendiol esters, flavonoids, steroids, tannin, quinines, coumarins, carotenoids, amino acids, polysaccharides, essential and volatile oils and many other chemical groups. *Calendula officinalis* exerted many therapeutic effects including antibacterial, antifungal, anthelmintic, antiviral, cytotoxic, antioxidant, anti-inflammatory, analgesic, hepatoprotective, cardioprotective, gastroprotective, wound healing and many other effects. The present review was designed to highlight the chemical constituents and the pharmacological and therapeutic effects of *Calendula officinalis*.

TAXONOMIC CLASSIFICATION

Kingdom: Plantae; **Subkingdom:** Tracheobionta; **Division:** Magnoliophyta; **Class:** Magnoliopsida; **Subclass:** Asteridae; **Order:** Asterales;

Family: Asteraceae; **Tribe:** Calenduleae; **Genus:** *Calendula*, **Species:** *C. officinalis*[47-48].

Common names

It was commonly known as Ekhwan asfar and Atherion makhzani (**Arabic**), Chin Chan Ts'ao (**Chinese**), African marigold, *Calendula*, Common marigold, Garden marigold, Marigold, Pot marigold (**English**), Butterblume (**German**), Zergul (**Hindi**), Galbinele (**Romanian**) and Ringblomma (**Swedish**) [49-50].

Distribution

The plant is native to Central and Southern Europe, Western Asia and the US [51].

Traditional uses

C. officinalis was used traditionally in the treatment of inflammations of internal organs, gastrointestinal ulcers and dysmenorrhea, as a diuretic and diaphoretic and for convulsions. It was also used for inflammations of the oral and pharyngeal mucosa, wounds and burns. *Calendula* tea was used as eyewashes, gargles, diaper rashes and other inflammatory conditions of the skin

and mucous membranes[53].

Part used

The flowers and the leaves are the chief parts which used medicinally. The essential oil from flowers was also used medicinally[48].

Description

C. officinalis is an annual or biennial plant attaining height of 30-60 cm. Leaves lower spatulate, 10-20 cm long and 1-4 cm wide; higher oblong and mucronate, 4-7 cm long; stem angular, hairy and solid; flower heads bright yellow to orange; marginal flowers in cultivated plants multi-seriate, corolla oblong spatulate, 15-25 mm long and 3 mm wide; corolla of disc flowers rounded, at the top tridentate, 1.5-2.5 cm long and 4-7 mm in diameter with 5 mm long tubular florets [54, 55].

Physiochemical parameter

Total ash: 10-14%, acid soluble ash: 10%, water soluble ash: 6%, acid-insoluble ash: not more than 2%, water-soluble extractive value: 20-21.6%, petroleum ether soluble extractive value: 1.6%, alcohol soluble extractive value: 2.4%, loss on drying: not more than 10% and crude fiber: 32% [56-57].

Chemical constituents:

The plant contained saponins, triterpene diol esters and flavonoids. The orange flower contained high carotenoids⁽⁵⁸⁻⁶¹⁾. The phytochemical screening of petroleum ether, chloroform, methanol and water extracts of *Calendula officinalis* leaf showed that petroleum ether extracts contained fatty acids, chloroform extracts contained triterpene and sterols. Flavonoids, carbohydrates, amino acids and saponins were present in methanol extract, while, saponins, phenolic substances and tannins were present in the water extract of *Calendula officinalis*⁽⁶²⁻⁶³⁾. However, in another study, petroleum ether extract showed the presence of carotenoids, steroids, saponins and tannin. Chloroform extract showed the presence of steroids, triterpene and tannin. Ethanolic extract showed the presence of alkaloids, flavonoids, and saponins. Aqueous extract showed the presence of flavonoids and saponins [58].

Quinones were isolated from different parts of *C. officinalis*. They were included plastoquinone, phylloquinone, α -tocopherol and ubiquinone [64]. Coumarins included scopoletin, umbelliferone and esculetin were isolated from the ethanol extract of the inflorescence of the *C. officinalis*. Many terpenoids were isolated from the petroleum ether extract of *C. officinalis* flowers, including calenduladiol-3-O-palmitate, calenduladiol-3-O-myristate, oleanolic acid saponins: calenduloside AH, oleanane triterpene glycoside: calendulaglycoside A, calendulaglycoside A6'-O-n-methyl

ester, calendulaglycoside A6'-O-n-butyl ester, calendulaglycoside B, calendulaglycoside B 6'-O-n-butyl ester, calendulaglycoside C, calendulaglycoside C 6'-O-n-methyl ester, calendulaglycoside C 6'-O-n-butyl ester, calenduloside F6-O-n-butyl ester, calenduloside G6-O-n-methyl ester, 3- monoesters of taraxasterol, Ψ -taraxasterol, lupeol, erythrodiol, brein, ursadiol, faradiol-3-O-palmitate, faradiol-3-O-myristate, faradiol-3-O-laurate, arnidiol-3-O-palmitate, arnidiol-3-O-myristate, arnidiol-3-O-laurate, glucosides of oleanolic acid I, II, III, VI, VII, glucuronides F, D, D2, C, B and A. and ester of oleanane[60, 66-81].

The amino acids in the leaves were about 5 %, in the stems 3.5 % and in the flowers 4.5 %. Fifteen amino acids were isolated from the ethanol extract of the flowers included alanine, arginine, aspartic acid, asparagine, valine, histidine, glutamic acid, leucine, lysine, proline, serine, tyrosine, threonine, methionine and phenylalanine [82].

Babae *et al.*, found that the total antioxidant, polyphenol and flavonoid and quercetin concentration of the 2% flowers extract were $2353.4 \pm 56.5 \mu\text{M}$, $313.40 \pm 6.52 \text{ mg/g}$, $76.66 \pm 23.24 \text{ mg/g}$, and $19.41 \pm 4.34 \text{ mg/g}$, respectively. However, Fonseca *et al.*, found that the total polyphenols, total flavonoids, rutin and narcissin contents of *Calendula officinalis* were 28.6 mg/g, 18.8 mg/g, 1.6 mg/g and 12.2mg/g, respectively. On the other hand, more flavonoids were isolated from *Calendula officinalis* included quercetin, isorhamnetin, isoquercetin, isorhamnetin-3-O- β -D-glycoside, rutin, isoquercitrin, neohesperidoside, isorhamnetin-3-O-neohesperidoside, isorhamnetin-3-O-2-rhamnosyl rutinoside, isorhamnetin-3-O-rutinoside, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, narcissin, calendoflaside, calendoflavoside and calendoflavobioside. Water-soluble polysaccharides reached (15%) included rhamnoarabinogalactans and arabinogalactans[51,737, 34, 85-86].

Calendula officinalis L. accumulated large amounts of carotenoids in its inflorescences. The yellow-to-orange color of inflorescences is mostly due to carotenoids. The carotenoid content and profile was investigated in four selected varieties of *Calendula*: Double Esterel Orange, Radio Extra Selected, Bonbon Abricot and Double Esterel Jaune. The carotenoid content was higher in orange varieties: 276 mg/100 g fresh flowers for Double Esterel Orange and 111 mg/100 g fresh flowers for Radio variety. All varieties contain the same pigments but there were significant differences for the ratio between individual pigments. Orange varieties contain higher amounts of hydrocarbons: 44.5% of total carotenoid as in Double Esterel Orange; while yellow varieties contain mostly oxygenated derivatives: 97% of total carotenoids as in Double Esterel Jaune. The main pigments identified were: flavoxanthin, lutein, rubixanthin, β -carotene, γ -carotene and lycopene [87-89]. The total oils extracted from the dried flowers of *Calendula officinalis* ranged

from 0.1 to 0.3% [51, 90-91]. The essential oil compounds isolated from *Calendula officinalis* flower were included: α -copaene, α -ionone, α -humulene, geranylacetone, γ -muurolene, β -ionone, ledene, α -muurolene, γ -cadinene, δ -cadinene, α -cadinene, α -calacorene, caryophyllene oxide, copaeen-4- α -ol, β -oplophenone, viridiflorol, ledol, 1,10-di-epi-cubenol, 1-epi-cubenol, epi- α -muurolol, α -cadinol and cadalene. The volatile fraction obtained from *Calendula officinalis* flowers were included α -cubebene, α -copaene, β -cubebene, α -gurjunene, β -cariophyllene, α -ionone, α -humulene, γ -muurolene, β -ionone, α -muuronele, γ -cadinene, δ -cadinene and α -cadinene [91].

However, the lipid content of seeds varied between 13.6 and 21.7 g oil/100 g seeds. The calendic and linoleic acids were the two dominant fatty acids in the total lipids (51.4 to 57.6% and 28.5 to 31.9% respectively). Polar lipids were also characterized by higher unsaturation ratios (with the PUFAs content between 60.4 and 66.4%), while saturates (consisted mainly of palmitic and very long-chain saturated fatty acids) [92].

Pharmacological effects

Antimicrobial and anthelmintic effects

The antimicrobial effect of ethanol crude extract of petals and reproductive parts of flowers in different concentrations was evaluated against eight types of bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterococcus pneumoniae*). The extracts of petals part were clearly superior against all bacteria especially *Pseudomonas aeruginosa* (inhibition zone was 25mm in the concentration of 100 mg/ml), and *Staphylococcus aureus* (inhibition zone was 14mm in the concentration 50mg/ml); while the extracts of reproductive parts were less effective than petals part [93].

The antimicrobial activity of methanol and ethanol extracts of *Calendula officinalis* petals was tested against clinical pathogens including bacteria and fungi. Methanol extract of *C. officinalis* exhibited better antibacterial activity against most of the tested bacteria, than ethanol extract. Both methanol and ethanol extracts showed excellent antifungal activity against tested strains of fungi [94-95].

The methanol extract and 10% decoction of the plant's flowers showed antimicrobial activity against facultative aerobic periodontal bacteria (*Porphyromonas gingivalis*, *Prevotella* spp., *Furobacterium nucleatum*, *Caphocytophaga gingivalis*, *Veilonella parvula*, *Eikenella corrodens*, *Peptostreptococcus micros* and *Actinomyces odontolyticus*) with MIC 2048 mg/l [96].

Mouthwashes containing *Calendula officinalis* reduced the number of microorganisms adhered to the sutures after extraction of unerupted third molars compared to the control group [97].

The antibacterial activities of free oleanolic acid and its glucosides and glucuronides isolated from marigold (*Calendula officinalis*) were investigated. Oleanolic acid inhibited bacterial growth and survival, influenced cell morphology and enhanced the autolysis of Gram-positive bacteria suggesting that bacterial envelopes are the target of its activity [98].

The essential oil of the flowers showed good potential antifungal activity (at 15 μ l/disc) when tested against *Candida albicans* (ATCC64548), *Candida dubliniensis* (ATCC777), *Candida parapsilosis* (ATCC22019), *Candida glabrata* (ATCC90030), *Candida krusei* (ATCC6258), and yeast isolated from humans [99].

Extracts of dried flowers from *Calendula officinalis* were examined for their ability to inhibit the human immunodeficiency virus type 1 (HIV-1) replication. Both organic and aqueous extracts were relatively nontoxic to human lymphocytic Molt-4 cells, but only the organic one exhibited potent anti-HIV activity in an *in vitro* MTT/tetrazolium-based assay. In addition, in the presence of the organic extract (500 micrograms/ml), the uninfected Molt-4 cells were completely protected for up to 24 h from fusion and subsequent death, caused by co-cultivation with persistently infected U-937/HIV-1 cells. It was also found that the organic extract from *Calendula officinalis* flowers caused a significant dose- and time-dependent reduction of HIV-1 reverse transcription (RT) activity. An 85% RT inhibition was achieved after a 30 min treatment of partially purified enzyme in a cell-free system [100].

A chloroform extract also inhibited HIV-1 reverse transcriptase activity in a dose-dependent manner (ED₅₀: 51.0mg/ml). A 5% hot aqueous extract of the flowers (2 ml) inhibited the replication of encephalitis virus after intraperitoneal administration to mice [101].

A tincture of the flowers suppressed the replication of herpes simplex, influenza A2 and influenza APR-8 viruses *in vitro* [102].

The methanolic and ethanolic extract of leaves of *Calendula officinalis* was prepared in three different concentrations 5, 10 and 15 mg/ml for investigation of anthelmintic activity *in vitro* against Indian adult earth worm, *Pheretima posthuma*. The results suggested that both the extracts showed significant anthelmintic activity as compared to the standard drug (albendazole 10 mg/ml), and it was also noticed that higher concentrations depicted better anthelmintic activity *in vitro* [103].

Glycosides of oleanolic acid isolated from marigold (*Calendula officinalis*) inhibited the development of L3 *Heligmosomoides polygyrus* larvae, the infective stage of this intestinal parasitic nematode. In addition, both oleanolic acid and its glucosides reduced the rate of L3 survival during prolonged storage, but only oleanolic acid glucuronides affected nematode infectivity [98].

The effects of saponins of *Calendula officinalis* on the infectivity of *Heligmosomoides polygyrus* was evaluated in mice. The immune activation provoked by

third-stage larvae exposed to *Calendula officinalis* glucuronides and the pattern of glycosylation of larval antigens which appeared to be crucial for induction of cytokine production in mice were examined; higher concentrations of IL-6, IFN- γ , IL-10 and TNF- α were observed in serum or intestine one week post infection. Three weeks later, in the chronic phase of infection, cells in culture were able to produce IL-6, IFN- γ , TNF- α and IL-17. Re-stimulation of cells with *H. polygyrus* antigen resulted in reduced production of IL-6, and TNF- α . The pattern of cytokine production co-existed with reduced expression of terminal glucose, α -linked mannose, N-acetyl-galactosamine, β -galactose, N-acetyl-glucosamine and α -fucose in several protein bands. Galactose, as a new terminal carbohydrate residue appeared in 20-24kDa protein bands. The number of immunogenic epitopes in parasitic antigens was reduced; only three protein bands of 56, 26 and 12kDa were recognized by IgG [104].

Wound and burn healing

The effects of oral and topical application of *Calendula officinalis* flower extract on excision wounds were checked in rats. The percentage of wound closure was 90.0% in the extract-treated group, whereas the control group showed only 51.1% on the eighth day of wounding ($P < 0.01$). The days needed for re-epithelialization were 17.7 for the control animals; while, extract treatment at a dose of 20 or 100 mg/kg bw reduced the period to 14 and 13 days, respectively. A significant increase was observed in the hydroxy proline and hexosamine content in the extract-treated group compared with the untreated animals⁽¹⁰⁵⁾.

Surgically induced skin wounds in rats were treated with a 5% *Calendula* ointment in combination with allantoin. The drug combination was found to markedly stimulate physiological regeneration and epithelialization. This effect was attributed to more extensive metabolism of glycoproteins, nucleoproteins and collagen protein during the regenerative period in the tissues [106].

Effect of *Calendula officinalis* flower extract was investigated against experimentally induced thermal burns in rats. Burn injury was made on the shaven back of the rats under anesthesia and the animals were treated orally with different doses of the flower extract (20 mg, 100 mg and 200 mg/kg body weight). The animals treated with the extract showed significant improvement in healing when compared with the control untreated animals. The indicators of the wound healing such as collagen-hydroxyproline and hexosamine contents were significantly increased in the treated group indicating accelerated wound healing in the treated animals. The acute phase proteins-haptoglobin and orosomucoid which were increased due to burn injury were found to be decreased significantly in 200 mg/kg body weight extract treated animals. The antioxidant defense mechanism, which was decreased in the liver during burn injury, was found to be enhanced in treated animals. The lipid

peroxidation was significantly lowered in the treated group when compared to control animals. Tissue damage marker enzymes (alkaline phosphatase, alanine and aspartate transaminases) were significantly lowered in the treated groups in a dose dependant manner. The histopathological analyses of skin tissue also gave the evidence of the increased healing potential of the extract after burn injury [107].

The therapeutic efficacy of marigold (*Calendula officinalis*) extract was investigated in the epithelialization of lower leg venous ulcers. Twenty-one patients with 33 venous ulcers out of 34 patients were treated with (*Calendula officinalis* ointment) which applied twice a day for 3 weeks. The second group was a control group that consisted of 13 patients with 22 venous ulcers. In the control group, saline solution dressings were applied to ulcers for the same period. In the experimental group the total surface of all the ulcers at the beginning of the therapy was 67,544 mm². After the third week the total surface of all the ulcers was 39,373 mm² (a decrease of 41.71%). In seven patients, complete epithelialization was achieved. In the control group the total surface of all the ulcers at the beginning of the therapy was 69,722 mm². After the third week the total surface of all the ulcers was 58,743 mm² (a decrease of 14.52%). In four patients, complete epithelialization was achieved. There was a statistically significant acceleration of wound healing in the experimental group ($p < 0.05$)[108].

Photoprotective effect

The photoprotective effect of the topical formulations containing marigold extract (ME) (*Calendula officinalis* extract) was studied in ultraviolet (UV) B irradiation-induced skin damage. The physical and functional stabilities, as well as the skin penetration capacity, of the different topical formulations were evaluated. In addition, the *in vivo* capacity to prevent/treat the UVB irradiation-induced skin damage in hairless mice and skin penetration capacity of the formulation was investigated. All of the formulations were physically and functionally stable. The gel formulation was the most effective for the topical delivery of ME, which was detected as 0.21 $\mu\text{g}/\text{cm}^2$ of narcissin and as 0.07 $\mu\text{g}/\text{cm}^2$ of the rutin in the viable epidermis. This formulation was able to maintain glutathione reduced levels close to those of nonirradiated animals, but did not affect the gelatinase-9 and myeloperoxidase activities which increased by exposure to UVB irradiation. In addition, gel formulation reduced the histological skin changes induced by UVB irradiation that appear as modifications of collagen fibrils [109].

The *in vivo* protective effect of *Calendula officinalis* extract against UVB-induced oxidative stress in the skin of hairless mice was evaluated by determining reduced glutathione (GSH) levels and monitoring the secretion/activity of metalloproteinases. An oral treatment

of hairless mice with 150 and 300 mg/kg of *Calendula officinalis* maintained GSH levels close to non-irradiated control mice. In addition, this extract affected the activity/secretion of matrix metalloproteinases 2 and 9 (MMP-2 and -9) stimulated by exposure to UVB irradiation [84].

Anti-inflammatory and analgesic effects

Calendula officinalis flower extract possessed significant anti-inflammatory activity against carrageenan and dextran-induced acute paw edema. Oral administration of 250 and 500 mg/kg body weight *Calendula* extract produced significant inhibition (50.6 and 65.9% respectively) in paw edema of animals induced by carrageenan and 41.9 and 42.4% respectively with inflammation produced by dextran. Administration of 250 and 500 mg/kg body weight *Calendula* extract produced an inhibition of 32.9 and 62.3% compared to controls, respectively in chronic anti-inflammatory model using formalin. TNF-alpha production by macrophage culture treated with lipopolysaccharide (LPS) was found to be significantly inhibited by *Calendula* extract. Moreover, increased levels of proinflammatory cytokines IL-1beta, IL-6, TNF-alpha and IFN-gamma and acute phase protein, C-reactive protein (CRP) in mice produced by LPS injection were inhibited significantly by the extract. LPS induced cyclooxygenase-2 (Cox-2) levels in mice spleen were also found to be inhibited by the extract treatment [110].

The hydroalcoholic plant extracts of *Calendula officinalis* suppressed the cell-free systems activities of 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2), the key enzymes in the formation of proinflammatory eicosanoids from arachidonic acid [65].

The inhibitory activity of nine oleanane-type triterpene glycosides isolated from *Calendula officinalis* was studied against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 microg/ear) in mice, all of the compounds, except 1, exhibited marked anti-inflammatory activity, with ID₅₀ values of 0.05-0.20 mg per ear [73].

The anti-inflammatory activity of the 3 main triterpene diol esters of marigold was tested against croton oil-induced edema of the ears in mice. Faradiol-3-myristic acid ester and faradiol-3-palmitic acid ester were found to have the same dose-dependent anti-inflammatory activity. The non-esterified faradiol was more active than the esters and had an equivalent effect on inflammation as an equimolar dose of indomethacin [68].

A dose of 1200 µg/ear of an aqueous-ethanol extract showed 20% inhibition in croton oil-induced mouse oedema. The activity was attributed to the presence of triterpenoids, the three most active compounds were the esters of faradiol-3-myristic acid, faradiol-3-palmitic acid and 4-taraxasterol [112, 113]. The analgesic effects of *Calendula officinalis* was evaluated in thermal pain

threshold in male rats. *Calendula officinalis* extract significantly increased the tail flick latency compared to the control group (P<0.05), indicating that the extract reduced pain threshold [114].

Antioxidant effects

The evaluation of the *in vitro* antioxidant activity of *Calendula officinalis* using different methodologies, showed a dose-dependent effect of *Calendula officinalis* against different radicals [84].

An extract of *Calendula officinalis* was evaluated for its antioxidant potential *in vitro* and *in vivo*. *Calendula officinalis* extract was found to scavenge superoxide radicals generated by photoreduction of riboflavin and hydroxyl radicals generated by Fenton reaction and inhibited *in vitro* lipid peroxidation. Extract scavenged ABTS radicals and DPPH radicals and IC₅₀ were 6.5 and 100 mg/ml, respectively. The extract also scavenged nitric oxide and the IC₅₀ was found to be 575 mg/ml. Extract also produced dose-dependent scavenging of nitric oxide in culture. The oral administration of *Calendula* extract inhibited superoxide generation in macrophages *in vivo* by 12.6% and 38.7% at doses of 100 and 250 mg/kg bw. Oral administration of *Calendula officinalis* to mice for 1 month significantly increased catalase activity. The extract produced significant increase in glutathione levels in blood and liver. Glutathione reductase was increased, whereas glutathione peroxidase was found to be decreased after administration of *Calendula* extract [115].

Propylene glycol extracts of the petals and flower heads assayed for antioxidant activity by lipid peroxidation, indicate that the extract of the petals was more potent than the flower head extract, based on analysis of plasma and urine malondialdehyde (MDA) and urine isoprostane concentrations [116].

A residual aqueous extract taken after extraction with 70% methanol extract with ether, chloroform, ethyl acetate and n-butanol showed antioxidant activity by liposomal lipid peroxidation-induced Fe²⁺ and ascorbic acid [117].

The antioxidant activity of the butanolic fraction (BF) of *Calendula officinalis* was studied *in vitro*. Superoxide radicals O⁻ and hydroxyl radicals OH are observed in decreasing concentrations in the presence of increasing concentrations of BF with IC₅₀ values of 1.0 ± 0.09 mg/ml and 0.5 ± 0.02 mg/ml, respectively, suggesting a possible free radical scavenging effect. Lipid peroxidation in liver microsomes induced by Fe²⁺/ascorbate was 100% inhibited by 0.5 mg/ml of BF (IC₅₀=0.15 mg/ml). Its total reactive antioxidant potential (TRAP) (in microM Trolox equivalents) was 368.14 ± 23.03 and its total antioxidant reactivity (TAR) was calculated to be 249.19 ± 14.5 microM [118].

The antiglycation ability of *C. officinalis* was studied, it showed minimum inhibitory concentration (MIC₅₀) of (270 microg/ml). The antioxidant potentials

were 26.10, 22.07 and 16.06% at 0.5 mg, 0.25 mg and 0.125 mg [119].

Effects on oral health

A case of recurrent exfoliative cheilitis (eighteen-year-old man with chronic dry scaling lesion on his lips) was treated with a standardized topical preparation of *Calendula officinalis* (10% ointment). The results showed that *Calendula officinalis* was a potential therapy for exfoliative cheilitis [120].

The effect of *Calendula officinalis* flowers extract mouthwash as oral gel (by maceration in ethanol 70% for a 72 hour period) was evaluated in radiation-induced oropharyngeal mucositis (OM) in patients with head-and-neck cancer. Forty patients with neck and head cancers under radiotherapy or concurrent chemoradiotherapy protocols were receive either 2% calendula extract mouthwash or placebo. Patients were treated with telecobalt radiotherapy at conventional fractionation (200 cGy/fraction, five fractions weekly, 30–35 fractions within 4–7 weeks). The oropharyngeal mucositis was evaluated by the oral mucositis assessment scale (OMAS). Calendula mouthwash significantly decreased the intensity of OM compared to placebo at week 2 (score: 5.5 vs. 6.8, $p = 0.019$), week 3 (score: 8.25 vs. 10.95, $p < 0.0001$) and week 6 (score: 11.4 vs. 13.35, $p = 0.031$) [83].

The therapeutic effect of *Calendula officinalis* gel was evaluated in oral mucositis induced by chemotherapy (5-FU) in hamster. In the groups received both chemotherapy and *Calendula officinalis* treatment, healing of oral mucositis was significantly improved both clinically and histopathologically ($P < 0.05$) in comparison to untreated group. The group received 5% *Calendula officinalis* gel demonstrated better results in comparison to the group treated with 10% *Calendula officinalis* gel [121].

The effects of *Calendula* on human gingival fibroblast (HGF) mediated collagen degradation and recombinant human matrix metalloproteinase (MMP) activity was studied. *Calendula* at 2-3% concentration completely inhibited the MMP-2 activity in the zymograms. Quercetin inhibited HGF-mediated collagen degradation at 0.005, 0.01 and 0.02%, and MMP-2 activity in a dose-dependent manner. *Calendula* inhibited HGF-mediated collagen degradation and MMP-2 activity more than the same correlated concentration of pure quercetin [122].

Mouthwashes containing *Calendula officinalis* reduced the number of microorganisms adhered to the sutures after extraction of unerupted third molars compared to the control group [97].

In studying the efficacy of *C. officinalis* in reducing dental plaque and gingival inflammation, plaque index (PI), gingival index (GI), sulcus bleeding index (SBI), and oral hygiene index-simplified (OHI-S). It appeared that *C. officinalis* induced statistically significant

reduction in the scores of PI, GI, SBI (except OHI-S) ($P < 0.05$) [123].

Cytotoxic and Immunological effects

C. officinalis tea exerted selective dose-dependent cytotoxic action against cancer cells. *C. officinalis* tea exerted highly selective antitumor effect especially to melanoma Fem-x cells⁽¹²⁴⁾. *Calendula officinalis* saponins were antimutagenic for benzo(a)pyrene with a dose effect relationship *in vitro*. They also showed cytotoxic and antitumor activity against mouse Ehrlich carcinoma [125-126].

The cytotoxicity of *Calendula officinalis* was evaluated in L929 and HepG2 cells with the MTT assay. Cytotoxicity experiments demonstrated that *Calendula officinalis* was not cytotoxic for L929 and HepG2 cells at concentrations less than or equal to 15 mg/ml. However, in concentrations greater than or equal to 30 mg/ml, the toxic effects were observed [84].

Fifteen compounds isolated from *Calendula officinalis* were evaluated against the Epstein-Barr virus early antigen (EBV-EA) activation induced by TPA, ten compounds exhibited moderate inhibitory effects (IC_{50} values of 471-487 mol ratio/32 pmol TPA). Furthermore, upon evaluation of the cytotoxic activity against human cancer cell lines *in vitro*, two triterpene glycosides exhibited potent cytotoxic effects against colon cancer, leukemia, and melanoma cells [73].

Barajas *et al.*, evaluated the dual and opposite effect of *Calendula officinalis* flower extract as a chemoprotector and promoter in rat hepatocarcinogenesis model. It was reported that a protective activity of the plant extract was noted at low doses, while the doses above 10 mg/kg increased altered hepatocyte foci. Such a dual effect is an example of the phenomenon of hormesis [127].

Three extracts of *Calendula officinalis* (heptane, ethyl acetate and methanol) were introduced to a human skin fibroblast (HSF) and human breast cancer cells (T47D) cultures. The ethyl acetate but not the heptane and methanol extracts in concentrations above 25 microg/ml stimulated cell proliferation and cellular metabolism by increase of mitochondrial dehydrogenase activity. However, concentrations exceeding 75 microg/ml have been found to be toxic for cells [86].

The anti-tumor and immunomodulatory activities of laser activated *Calendula officinalis* extract (LACE) was investigated *in vitro*. Tumor cell lines derived from leukemias, melanomas, fibrosarcomas and cancers of breast, prostate, cervix, lung, pancreas and colorectal were used. The tumor cell proliferation *in vitro* was measured by BrdU incorporation and viable cell count. Effect of (LACE) on human peripheral blood lymphocyte (PBL) proliferation *in vitro* was also analyzed. Studies of cell cycle and apoptosis were performed in LACE-treated cells. *In vivo* anti-tumor activity was evaluated in nude mice

bearing subcutaneously human Ando-2 melanoma cells. The LACE extract showed a potent *in vitro* inhibition of tumor cell proliferation when tested on a wide variety of human and murine tumor cell lines. The inhibition ranged from 70 to 100%. Mechanisms of inhibition were identified as cell cycle arrest in G0/G1 phase and Caspase-3-induced apoptosis. The same extract showed an opposite effect when tested on PBLs and NKL cell line, in which *in vitro* induction of proliferation and activation of these cells was observed. The intraperitoneal injection or oral administration of LACE extract in nude mice inhibited *in vivo* tumor growth of Ando-2 melanoma cells and prolonged the survival day of the mice [128].

The polysaccharides isolated from an aqueous extract of Flos Calendulae enhanced phagocytosis in human granulocytes *in vitro* in the colloidal carbon clearance test. The polysaccharides isolated from flowers aqueous extract also enhanced phagocytosis when administered (10 mg/kg bw) intraperitoneally to mice. On the other hand, intraperitoneal administration of unsaponifiable fraction (0.5 ml) of a hydroalcoholic extract of the flowers also stimulated phagocytosis in mice inoculated with *Escherichia coli* [85, 87, 129].

Genotoxic and anti-genotoxic effects

The induction of unscheduled DNA synthesis (UDS) in rat and reversion of diethylnitrosamine (DEN)-induced UDS was determined for four different flower *C. officinalis* extracts [10 mg of solid material per ml of aqueous (AE), aqueous-ethanol (AEE), ethanol (EE) and chloroform (CE)]. In the UDS assay in liver cell cultures, DEN at 1.25 microM produced a maximal increase of 40% (³H-thymidine ((³H)TdR) incorporation, and both, AE and AEE showed complete reversion of the DEN effect at around 50 ng/ml and between 0.4 to 16 ng/ml, respectively. In the absence of DEN, these two polar extracts induced UDS at concentrations of 25 microg for AE and 3.7 microg/ml for AEE to 100 microg/ml in rat liver cell cultures. Concentrations producing genotoxic damage were three orders of magnitude above concentrations that conferred total protection against the DEN effect. Thus, at the lower end, ng/ml concentrations of the two polar extracts AE and AEE conferred total protection against the DEN effect and at the higher end, g/ml concentrations produced genotoxic effects [130].

Effects on stress and excitotoxic brain damage

The neuroprotective effect of *Calendula officinalis* Linn. flower extract (COE) on Monosodium glutamate (MSG)-induced neurotoxicity was evaluated in rats. Adult Wistar rats were administered systemically for 7 days with MSG and after 1h of MSG injection, rats were treated with COE (100 and 200 mg/kg) orally. At the end the treatment period, animals were assessed for locomotor activity and were sacrificed; brains were isolated for estimation of LPO, GSH, CAT, TT, GST, Nitrite and for

histopathological studies. MSG caused a significant alteration in animal behavior, oxidative defense (raised levels of LPO, nitrite concentration, depletion of antioxidant levels) and hippocampal neuronal histology. Treatment with COE significantly attenuated behavioral alterations, oxidative stress, and hippocampal damage in MSG-treated animals [131].

The neuroprotective effect

The neuroprotective effect of *Calendula officinalis* flower extract (COE) on 3-NP-induced neurotoxicity in rats was evaluated by observing behavioral changes, OS and striatal damage in rat brain. Adult female Wistar rats were pretreated with vehicle or COE (100 and 200 mg/kg) for 7 days, followed by cotreatment with 3-NP (15 mg/kg, intraperitoneally) for the next 7 days. At the end of the treatment schedule, rats were evaluated for alterations in sensory motor functions and short-term memory. Animals were sacrificed and brain homogenates were used for the estimation of lipid peroxidation (LPO), glutathione, total thiols, glutathione S-transferase, catalase and nitrite. A set of brain slices was used for the evaluation of neuronal damage in the striatal region of the brain. 3-NP caused significant alterations in animal behavior, oxidative defense system evidenced by raised levels of LPO and nitrite concentration, and depletion of antioxidant levels. It also produced a loss of neuronal cells in the striatal region. Treatment with COE significantly attenuated behavioral alterations, oxidative damage and striatal neuronal loss in 3-NP-treated animals [132].

Cardiovascular effect

Calendulozide B-trioside isolated from rhizomes of *Calendula officinalis* didn't have cardiovascular effects, didn't affected the tone of intestinal smooth muscles, didn't affected the diuretic renal function and electrolytes excretion in urine and didn't affected the biligenic function of the liver. It was devoid of locally irritation properties, but with low hemolytic activity (15000 after Kofler) and an insignificant toxicity both with its one-time and chronic administration [133].

The cardioprotective effect of *Calendula officinalis* in ischemic heart disease was evaluated. The treated rat hearts were perfused with calendula solution at 50 mM in KHB buffer (in mM: sodium chloride 118, potassium chloride 4.7, calcium chloride 1.7, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulfate 1.2, and glucose 10) for 15 min prior to subjecting the heart to ischemia, while the control group was perfused with the buffer only. Calendula achieved cardioprotection by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocyte apoptosis. Cardioprotection appears to be achieved by changing ischemia reperfusion-mediated death signal into a survival signal by modulating

antioxidant and anti-inflammatory pathways as evidenced by the activation of Akt and Bcl2 and depression of TNF α [134].

Gastrointestinal effects

Calendulozide B-trioside, isolated from rhizomes of *Calendula officinalis*, in doses of 5, 10, 20 and 50 mg/kg exerted an antilcerous action in 3 experimental ulcer models of different genesis (caffein-arsenic, butadion and ligation of pylorus) and also displayed a certain antiphlogistic and sedative action [87]. The influence of *Calendula officinalis* on heparin-binding epidermal growth factor (HB-EGF)-like growth factor gene expression in KATO-III cells under the stimulation of *H. pylori* strain N6 using real-time PCR was investigated with and without addition of and *Calendula officinalis*. Addition of *Calendula officinalis* led to a significant reduction of *H. pylori* induced increase in gene expression of HB-EGF (reduced to 75.32 \pm 1.16% vs. control; p<0.05) [135].

170 patients with duodenal ulcers and/or gastroduodenitis, treated with a herbal combination containing calendula showed improvement of symptoms in 90%⁽¹³⁶⁾. 24 adults with non-specific colitis treated with herbal tea included calendula, showed improved symptoms in 96% of the patients within two weeks [137].

The hepatoprotective effect

The hepatoprotective effect of calendula flowers and/or thyme leave extracts on aflatoxins (AFs)-induced oxidative stress, genotoxicity and alteration of p53 and bcl2 gene expressions were evaluated. Animals treated with the extracts 1 week before AFs treatment showed a significant decrease in oxidative damage markers, micronucleated cells, DNA fragmentation and modulation of the expression of pro-apoptotic genes [138]. The hydroalcohol extract of the flowers, when given to CCl₄-intoxicated liver in albino male wistar rats at a dose of 10 ml/kg, resulted in a reduction of hepatocytolysis by 28.5 % due to reduction in glutamo-oxalate-transaminase (GOT) and glutamo-pyruvate-transaminase (GPT).

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Histoenzymology showed reduction of steatosis of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), cytochromoxidase (Cyox) and Mg²⁺-dependant adenosine triphosphatase (ATPase)[139]. The hot water extract of *C. officinalis* flowers exhibited antihepatoma activity against five human liver cancer cells - HepG2/C3A, SK-HEP-1, HA22T/VGH, Hep3B and PLC/PRF/5 – with an inhibitory effect of 25- 26% at a dose of 2000 μ g/ml [140].

Other effects

Calendula officinalis flowers extracts exerted estrogenic activity in ovariectomized animals [63, 141, 142]. *Calendula officinalis* saponosides extracts have mild sedative effects and synergistic effects with sedative medications such as barbiturates [143]. Aqueous alcoholic extract of florets also showed CNS inhibitory effect with marked sedative activity in experimental animals [63].

Adverse effects and toxicity

There is a low potential for sensitization after frequent skin contact with the drug. A low rate of contact dermatitis (less than 1%) occurred in patients patch-tested with a tincture of 10% Calendula. However, only 2 of 1032 patients had a positive skin reaction to Calendula [144].

Dose

1 to 2 grams of *Calendula* powder in one cup of water. Wound treatment, ointment 2% to 5%, apply topically to the affected area [51, 145]. Tincture (1:9 in 20% alcohol): 2-4 ml per ¼-½ cup of water. Tincture (1:5 in 90% alcohol): 0.3-1.2 ml three times daily [146-147].

CONCLUSION

Calendula officinalis is a plant with wide range of chemical constituents which exerted many pharmacological effects. There is a great promise for development of novel drugs from *Calendula officinalis* to treat many human diseases as a result of its effectiveness and safety.

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