



(REVIEW ARTICLE)



A review on *Medicago sativa*: A potential medicinal plant

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International Journal of Biological and Pharmaceutical Sciences Archive, 2021, 01(02), 022–033

Publication history: Received on 13 January 2021; revised on 01 February 2021; accepted on 03 February 2021

Article DOI: <https://doi.org/10.30574/ijbpsa.2021.1.2.0302>

Abstract

Medicago sativa (Family: Fabaceae) was used traditionally for the treatment of arthritis, kidney problems, fever, as diuretic, anti-cancer, anti-rheumatic, cardiogenic, depurative, lactagogue, emmenagogue, antiscorbutic and in the treatment of boils. Leaves and sprouts were also consumed as vegetable salad. The leaves or seeds were sold as bulk powdered herb, capsules, and tablets for nutritional supplement in health food stores. The phytochemical analysis of *Medicago sativa* showed the presence of proteins, carbohydrates, saponins, lignin, phenolic compounds, tannins, alkaloids triterpene glycosides, carotenoids, sterols, phytoestrogens, flavones, isoflavonoids and phenolic compounds. The previous pharmacological investigation showed that the plant possessed antioxidant, antidiabetic, reproductive, anti-inflammatory, antimicrobial, dermatological, anxiolytic, hepatoprotective, neuroprotective, immunological, cardioprotective, cytotoxic, anti-scorbutic, anti-anemic, xanthine oxidase inhibition and many other pharmacological effects. The current review discussed the bioactive constituents and pharmacological activities of *Medicago sativa*.

Keywords: *Medicago sativa*; Constituents; Traditional uses; Pharmacology; Therapeutic; Toxicology

1. Introduction

Medicinal plants are the Nature's gift to human beings to help them pursue a disease-free healthy life. Plants have been used as drugs by humans since thousands of years ago. As a result of accumulated experience from the past generations, today, all the world's cultures have an extensive knowledge of herbal medicine. The phytochemical analysis of *Medicago sativa* showed the presence of proteins, carbohydrates, saponins, lignin, phenolic compounds, tannins, alkaloids triterpene glycosides, carotenoids, sterols, phytoestrogens, flavones, isoflavonoids and phenolic compounds. The previous pharmacological investigation showed that the plant possessed antioxidant, antidiabetic, reproductive, anti-inflammatory, antimicrobial, dermatological, anxiolytic, hepatoprotective, neuroprotective, immunological, cardioprotective, cytotoxic, anti-scorbutic, anti-anemic, xanthine oxidase inhibition and many other pharmacological effects. The current review was designed to discuss the bioactive constituents and pharmacological activities of *Medicago sativa*.

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1.1. Synonyms

Medica sativa, *Medicago afganica*, *Medicago asiatica subsp. sinensis*, *Medicago beipinensis*, *Medicago grandiflora*, *Medicago ladak*, *Medicago mesopotamica*, *Medicago orientalis*, *Medicago polia*, *Medicago praesativa*, *Medicago praesativa subsp. spontanea*, *Medicago sativa f. alba*, *Medicago sativa var. grandiflora*, *Medicago sativa subsp. sativa*, *Medicago sativa f. sativa*, *Medicago sativa var. tibetana*, *Medicago sogdiana*, *Medicago tibetana*, *Trigonella upendrae* [1].

1.2. Taxonomic classification

Kingdom: Plantae, Subkingdom: Viridiplantae, Infrakingdom: Streptophyta, Superdivision: Embryophyta, Division: Tracheophyta, Subdivision: Spermatophytina, Class: Magnoliopsida, Order: Fabales, Family: Fabaceae, Genus: *Medicago*, Species: *Medicago sativa* [2].

1.3. Common names

Arabic: burseem, jatt; English: alfalfa, lucerne; French: alfalfa, lucerne, luzernecultivee; Germany: blaueluzerne, luzerne, saatluzerne; Hindi: lasunghas, rizka, wilayati-gawuth; Italian: erbamedica, medica; Korean: jajukgaejari; Russian: lyutzernaposevnaya, lyutzernasinyaya; Spanish: alfalfa rustica, mielga; Swedish: blalusern [3-4].

1.4. Distribution

It is native to Africa (Algeria, Libya, Morocco, Tunisia), Asia (Afghanistan, Cyprus, Iran, Iraq, Palestine, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Russian Federation, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Mongolia, China, Korea, Pakistan), Europe (Denmark, Ireland, Norway, Sweden, United Kingdom, Austria, Belgium, Czechoslovakia, Germany, Hungary, Netherlands, Poland, Switzerland, Belarus, Estonia, Latvia, Lithuania, Moldova, Russian Federation-European part, Albania, Bulgaria, Former Yugoslavia, Greece, Italy, Romania, France, Portugal, Spain). It is naturalized in Africa (Egypt, South Africa), Asia (India, Nepal, Sri Lanka), Australasia (Australia, New Zealand), Europe (Finland), Northern America (United States), Southern America (Brazil, Ecuador, Peru, Argentina, Chile, Uruguay) and it is widely cultivated [5].

1.5. Traditional uses

Medicago sativa sprouts were consumed as vegetable salad. Its leaves or seeds were also sold as bulk powdered herb, capsules, and tablets for nutritional supplement in health food stores [5]. It was used traditionally as ayurvedic and homoeopathic medicine in central nervous system disorders, kidney pain, diabetes, inflammation and obesity [6-7].

Alfalfa sprouts or leaves were also used traditionally for the treatment of arthritis, kidney problems, fever, as anti-cancer, anti-rheumatic, cardiogenic, depurative, lactagogue, emmenagogue, antiscorbutic and in the treatment of boils [8].

In South America the plant was used as diuretic and in the treatment of kidney and vesicular swelling [9].

1.6. Parts used traditionally

Leaves, roots and seeds [5-9].

1.7. Chemical constituents

The preliminary phytochemical analysis of alfalfa seed extracts showed the presence of proteins, carbohydrates, saponins, lignin, phenolic compounds, tannins, alkaloids triterpene glycosides, carotenoids, sterols, phytoestrogens (cumestrol), flavones, isoflavonoids and phenolic compounds [10-14].

The moisture, protein, fat, fiber and ash in the green forage were: 80, 5.2, 0.5, 3.5 and 2.4 g/100g, in the whole meal were: 7.5, 16, 2.5, 27.3 and 9.1 and g/100g, and in the leaf meal were: 8, 20.4, 2.6, 17.1 and 11.5 g/100g respectively [15].

The composition of the protein-xanthophyll extract from alfalfa (*Medicago sativa*) was included: protein 45-60%, fat 9-11%, simple sugars (soluble dietary fiber) 1-2%, polysacharydes (insoluble dietary fiber, including cellulose 2-3%) 11-15%, saponins \leq 1.4%, minerals 8-13%, coumestrol \leq 100 mg/kg, phytynians \leq 200 mg/kg, isoflavones \leq 350 mg/kg, and L-canavanine \leq 4.5 mg/kg [11].

Phytoestrogenic compounds, coumestrol, loliolide, liquiritigenin, isoliquiritigenin, and (4*S*,6*S*)- and (4*R*,6*S*)-4-hydroxy-6-pentadecyltetrahydropyr-2-one were isolated from *Medicago sativa* [16].

The pharmacologically active substances present in *Medicago sativa* include alkaloids (stachydrine, homostachydrine), aminoacids (arginine, asparagine, cystine, histidine, isoleucine, leucine, methionine, tryptophan, valine), coumarins (medicagol, sativol, trifoliol, lucernol, 4-*o*-methyl coumesterol, 3-methoxycoumesterol, 11,12-dimethoxy-7-hydroxyl coumesterol), flavonoids (quercetin, myricetin, luteolin, apigenin, chrysoeriol, tricetin, coumestrol, biochanin A, genistein), saponins, steroids (stigmasterol, campesterol, cycloartenol, β -sitosterol), volatile components (nonadienal, benzaldehyde, 2-methyl 4-pentenol, terpenes, limonene, linalool, *trans*-ocimene, furanoids, ethyl benzaldehyde, butanol, hexanol, octanol, alcohols, pentan-3-ol, 3-methylbutanol, *trans*-2-pentenol, *trans*-2-hexenol, *trans*-3-hexenol, pent-1-en-3-ol, oct-1-en-3-ol, octa-1,5-dien-3-ol, benzyl alcohol, 2-phenylethanol, ketones, pent-1-en-3-one, pentan-3-one, octan-3-one, methyl phenyl ketone, esters, *trans*-3-hexenylacetate, *trans*-3-hexenylbutanoate, aldehydes, hexanal, *trans*-2-pentenol, *trans*-2-hexenal, *trans*-2-nonenal, *trans*-2,4-hexadienal, furane-2-ethyl), acids (lauric, maleic, malic, malonic, myristic, oxalic, palmitic, quinic), purines (adenine, guanine, xanthine, hypoxanthine), canavanine, amino acids (medicanine, lysine, arginine, histidine, tyrosine, phenylalanine, methionine, aspartic acid, glutamic acid, asparagine, serine, alanine, threonine), vitamins (A, B₁, B₆, B₁₂, C, D, E, K), ketones (myristone, alfalzone) and other constituents such as fructose, pectin, chlorophyll, minerals and trace elements [17-20].

The total saponin content was (2.12 μ mol/g of dry matter at the beginning of germination and 6 μ mol/g after 8–16 days of seedling growth) [21]. Alfalfa contained more than 33 different saponins, they were tri-terpenoids composed of a thirty carbons, aglycone linked to one or more sugar moiety [22].

Saponins compounds such as medicagenic acid, 3-*o*-glucoside and 3,28-diglucoside of medicagenic acid, hederagenin, hederagenin glycosides, zanhic, bayogenin acid, azukisaponin and soyasapogenols A-I, were isolated from the aerial parts of alfalfa [22-24].

The total phenolics in the leaves extract of *Medicago sativa* was 37.0 \pm 0.02 mg gallic acid equivalent (GAE)/g dry matter, and total flavonoids was 12.62 \pm 0.17 mg rutin equivalent/g dry matter⁽⁶⁾. While, the total phenolic content of *Medicago sativa* flowers methanolic extract was 263.5 \pm 1.02 mg GAE/100g of dry weight lyophilized extract [25].

Flavonoids isolated from the aerial parts of *Medicago sativa* were included: (-)-medicarpin, (-)-mellelitolcarpan E, millepurpan, genistein, sissotrin, apigenin, luteolin, chrysoeriol, tricetin and acylated apigenin, luteolin, and tricetin. Furthermore, glycosides of the same flavonoids were also isolated from the aerial parts of the plant [18, 26-29].

Vitamins and minerals contents of the leaves (per 10 g) were: β -carotene: 380 \pm 191 mg, vitamin E: 9.9 \pm 1.9, vitamin C: 6 retinol activity equivalents, Fe: 5.4 \pm 0.8 mg, folic acid: 13.4 \pm 3.4 mg, Ca: 338 \pm 43 mg, Cu: 76 \pm 10 mg, Mn: 0.6 \pm 0.0 mg and Zn: 0.2 \pm 0.0 mg [30].

2. Pharmacological effects

2.1. Antioxidant effect

The antioxidant activity of *Medicago sativa* flowers extracts was assayed using the radical DPPH. The results revealed that aqueous was better than acetic acid and methanol for extracting bioactive compounds, in particular for total phenolic compounds from the flowers of alfalfa. The average content of active molecules was 263.5 \pm 1.02 mg GAE/100g of dry methanol extract weight. The phenolic content was correlated with the radical decomposing activity. However, all extracts showed antioxidant activity, but the water extract was more potent than the acetic and the methanol extracts. The order of inhibition (IC₅₀) were: water extract (0.924 mg/ml) > acetic acid extract (0.154 mg/ml) > methanol (0.079 mg/ml) [25].

The *in vitro* free radical scavenging activities of various extracts of *Medicago sativa* raw seeds and germinated seeds were carried out using DPPH, hydrogen peroxide and hydroxyl radicals, superoxide anion radicals and nitric oxide radicals tests. *Medicago sativa* raw seeds and germinated seedsethanolic extracts, scavenged the free radicals in a concentration dependent manner. The antioxidative effect was more prominent in *Medicago sativa* germinated seeds ethanolic extract [14].

Investigation of antioxidant activity of Alfalfa by DPPH and ferric reducing antioxidant power (FRAP) methods showed 54.42 and 56.71% inhibition of free radicals at the concentration of 250 μ g/ml of leaves crude extract, respectively. NO inhibition assay revealed 50.99% inhibitory activity of NO at the concentration of 250 μ g/ml [6].

Free radicals scavenging activity and antioxidant effect of the alcoholic extract of was studied *in vitro* using DPPH radical scavenging, ABTS radical scavenging, iron chelating activity, lipid peroxidation assay, nitric oxide scavenging assay, and alkaline DMSO assay. The results revealed that IC₅₀ values of antioxidant activity were 100.38 µg/ml (DPPH radical scavenging), 12.33 µg/ml (ABTS radical scavenging), 115.79 µg/ml (Iron chelating activity), 49.06 µg/ml (lipid peroxidation), 21.77 µg/ml (nitric oxide scavenging) and 15.91 µg/ml (alkaline DMSO) [8].

The antioxidant effect was investigated for extracts from different parts of *Medicago sativa* obtained by maceration, ultrasound-assisted extraction, accelerated solvent extraction, and supercritical fluid extraction. The extract from leaves obtained by supercritical fluid extraction showed the highest total flavonoid content (139.0 ± 7.1 mg rutin equivalents/g dry matter) [31].

Evaluation of the antioxidant activity of polysaccharides from alfalfa *in vitro* suggested that the polysaccharides had good antioxidant effect, especially scavenging activity for hydroxyl radical and DPPH radical [32].

2.2. Antidiabetic effect

The effect of the aqueous ethanol extract of *Medicago sativa* was studied on the blood sugar and histopathological changes in streptozotocin induced diabetic rats. The results revealed that blood sugar of the diabetic rats was significantly reduced by the extract. The final body weight in the diabetic control and extract treated rats increased significantly in comparison with the first body weight but not in the diabetic + extract group. The kidney weight in the diabetic + extract rats was increased significantly compared to the control + extract group. The total volume of kidney and cortex were similar in all groups, while the volume of medulla increased significantly in the diabetic + extract group compared to the control + extract group. Furthermore, the total glomerular volume increased significantly in diabetic rats compared to the control [33].

The hypoglycemic effect of *Medicago sativa* aqueous extract for 4 weeks was investigated in streptozotocin induced type 2 diabetic rats. *Medicago sativa* extract decreased postprandial glycaemia in type 2 diabetic and non-diabetic rats. The effect was mediated by enhancing insulin secretion by the extract [34].

The effects of aqueous extract of alfalfa (250 and 500 mg/kg for 21 days) on blood glucose and serum lipids were investigated in alloxan-induced diabetic rats. The aqueous extract decreased serum glucose, cholesterol, triglycerides, and LDL levels significantly in the diabetic rats and increased HDL levels. ALT and AST levels were also decreased. Histological investigation revealed that the aqueous extract caused reconstruction of damaged liver and enhanced Langerhans islets' diameter in pancreas [35].

2.3. Reproductive effects

Serum oestradiol levels, ovaries and uteri weights were significantly increased with the using of 9, 18 and 36mg /kg of alfalfa ethanolic extracts, for 15 days in female rats [36].

Plasma luteinizing hormone (LH) concentration was determined in ewes fed alfalfa. The peak LH level in control ewes was 40.1 ± 5.5 ng/ml, it was lower ($P < 0.05$) than in ewes fed phyto-estrogenic alfalfa (66.0 ± 16.8 ng/ml). Furthermore, the LH peak occurred later ($P < 0.05$) in the estrus period of ewes fed phyto-estrogenic alfalfa (15.4 ± 4.5 h) [37].

The activity of phytoestrogenic compounds (coumestrol, liquiritigenin, isoliquiritigenin, loliolide, and (4S,6S)- and (4R,6S)-4-hydroxy-6-pentadecyl tetrahydropyr-2-one) isolated from *Medicago sativa* was tracked by a transactivation assay for ER α and ER β . The tested compounds revealed higher transactivation via ER β compared with ER α . Loliolide, isoliquiritigenin, and (4S,6S)- and (4R,6S)-4-hydroxy-6-pentadecyl tetrahydropyr-2-one, but not coumestrol, preferentially inhibited 1 nM E₂ induced ER α activation, compared to ER β activation [16].

The effect of aerial parts aqueous extract of the of a mixture of *Medicago sativa* and *Salvia officinalis* on the reproductive system of mature female was studied in mice. The aqueous extract of the plants mixture was given orally with water supplement for two different periods (two and four weeks) and with two different doses (100 and 200 mg/kg/ day). A significant increase in body weight in all treated groups and an increase in reproductive organs weight especially in the groups received higher doses were detected. LH and estradiol levels at the estrus phase were significantly increase, while FSH was decreased in all treated groups. The histological examination showed remarkable increase in the number of ovarian follicles and corpora lutea. There was an increase in endometrial glands diameter especially in groups received the extract for long duration, while the uterine epithelial cells height was increased significantly in all treated groups [38].

The effects of *Medicago sativa* (ethanolic extract, 250 and 500 mg/kg bw for 22 days) on body and organs weights, serum estradiol, progesterone, total proteins, total cholesterol, the liver and kidney functions were studied in immature female rats. Body weight of rats of control group was higher compared to the rats given low dose of the extract (114.40 ± 5.35 versus 93.20 ± 7.57 g). Mean ovarian weight was significantly higher in rats of both doses (18.80 ± 2.94 mg for low dose and 22.80 ± 2.94 mg for high dose) compared to the control group. Serum progesterone levels were higher (49.04 ± 6.67 and 40.20 ± 11.92 ng/ml) for low and high dose, respectively. Follicular development, ovulation and corpus luteum formation were increased by the estrogen-like activity of the plant extract. A significantly higher serum total proteins in rats was recorded in high dose (65.01 ± 4.15 g/l) compared with control group and low dose group. There were no differences in the liver or kidney weights, serum urea concentrations and ALT activities of the treated rats [39].

2.4. Antiinflammatory effect

The anti-inflammatory potential of alfalfa was studied using lipopolysaccharide (LPS)-stimulated immune responses. The aerial parts chloroform extract inhibited immune responses stimulated by LPS more than ether, butanol, or water soluble extracts. 1 μ g/ml of LPS increased the concentrations of nitrite up to 44.3 μ M in macrophages, but it was reduced to 10.6 μ M by adding 100 μ g/ml chloroform extract. LPS treatment also increased the concentrations of TNF- α , IL-6, and IL-1 β to 41.3, 11.6, and 0.78 ng/ml in culture supernatants of the cells, but these cytokine levels decreased to 12.5, 3.1, and 0.19 ng/ml, respectively, by pre-treating with 100 μ g/ml of the extract. Mice injected with LPS (30 mg/kg bw) alone showed a 0% survival rate after 48 h of the injection, but 48-h survival of the mice increased to 60% after oral administration of the extract. Subfractions of the chloroform extract markedly suppressed LPS-mediated activation of the extracellular signal-regulated kinase and nuclear factor kappa-B [40].

Supplementation with *Medicago sativa* sprout ethyl extract inhibited the production of pro-inflammatory cytokines and alleviated acute inflammatory hazards in mice. The extract significantly reduced IL-6 and IL-1 β production and the NF-kappa B trans-activation activity of mitogen-stimulated RAW264.7 cells. Furthermore, the extract showed significantly lower serum TNF- α , IL-6, and IL-1beta levels at 9 hr after LPS challenge, and significantly higher survival rates than the control group [5].

The leaves extracts of *Medicago sativa* (7.8 to 500 μ g/ml) were examined for their inhibitory activity on NO released in RAW 264.7 cells, stimulated by bacterial LPS and IFN- γ . The extract possessed moderate anti-inflammatory activity, the 50% of the NO production by the induced RAW 264.7 cells was inhibited at the concentration of 147.24 μ g/ml of Alfalfa crude extract [6].

2.5. Hypolipidemic effect

The increased serum cholesterol and LDL levels were reduced by 38- 41.7% and 48- 53.3% respectively, in rabbits fed with alfalfa seed extract from the beginning or in established cholesterol fed hyperlipidaemic model. LDL lowering effect was maximum (64.4%) in a model fed with alfalfa meals without cholesterol [41].

The hypolipidemic effects of *Medicago sativa* sprouts were investigated in streptozotocin induced diabetes. The administration of methanol extract (500 mg/kg), petroleum ether (32.5mg) and butanol fractions (60 mg) for 4 weeks, significantly decreased triglycerides, total cholesterol, LDL and VLDL in comparison to rovastatin. Petroleum ether fraction exhibited the best antihyperlipidemic activity (12.23%). While, ethyl acetate fraction showed the best hypoglycemic activity in comparison with metformin. Histological improvement of the liver, pancreas and spleen confirmed the biochemical results [18].

The effects of alfalfa plant and sprout and saponin-free alfalfa plant on diet-induced liver cholesterol accumulation, bile acid excretion, and jejunal and colonic morphology were studied. Alfalfa plant saponins bound significant quantities of cholesterol both from ethanol solution and from micellar suspension. Alfalfa sprout saponins interacted with cholesterol to a lesser but significant extent. Bile acid adsorption was greatest for alfalfa plant and was not reduced by removal of saponins from the plant material. The ability of alfalfa to reduce liver cholesterol accumulation in cholesterol-fed rats was enhanced by removal of saponins and alfalfa sprouts did not prevent accumulation. Removal of saponins from alfalfa reduced the changes in intestinal morphology previously reported, but interaction with membrane cholesterol did not appear to be the cause of this effect of saponins [42].

Fifteen patients with hyperlipoproteinemia types IIA, IIB and IV were given 40 g of heat prepared alfalfa seeds 3 times daily at meal times for 8 weeks with otherwise unchanged diet. Body weight increased slightly during the first 4 weeks of alfalfa treatment probably because of the caloric content in the alfalfa seeds. However, the treatment significantly decrease the total and LDL cholesterol [43].

2.6. Antimicrobial effect

The petroleum ether, chloroform, benzene, methanol, ethanol and water extracts of *Medicago sativa* was evaluated for antibacterial activity, against *Staphylococcus aureus*, *Streptococcus pyogenes* MTCC 1928, *Proteus mirabilis* MTCC 425, *Escherichia coli* MTCC 2961, *Pseudomonas aeruginosa* MTCC 4676, *Klebsiella pneumoniae* MTCC 432 and *Salmonella typhi* MTCC 733. Methanol extract possessed significant activity against all the tested bacteria followed by chloroform and ethanol extract. Benzene and petroleum ether extracts did not show any significant activity. The antibacterial activity is more significant in solvent extracts compared to aqueous extract [7].

The antimicrobial effect of aqueous extract of alfalfa seed was studied against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*. It showed moderate activity against Gram positive, but not Gram negative microorganisms [44].

Medicago sativa root extract was tested for antibacterial activity against *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. The MIC of *Medicago sativa* root extract against *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*, was 125 mg/ml. The diameter of the inhibition zone was 16 mm against *Moraxella catarrhalis*, 13 mm against *Streptococcus pneumoniae* and 10 mm against *Haemophilus influenzae*, while *Staphylococcus aureus*, showed no sensitivity to the extract [45].

The antimicrobial activity of saponins from *Medicago sativa*, was studied against medically important yeasts, Gram positive and Gram negative bacteria. It possessed high activity against Gram positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, and *Enterococcus faecalis*) [46].

Antibacterial activity of *Medicago sativa* leaves extract was studied against 3 bacteria. The zones of inhibition of the petroleum ether and methanol extracts against *Escherichia coli* were 21 and 23, *Pseudomonas aeruginosa* 18 and 22 and *Staphylococcus aureus* 19 and 23mm, respectively [47].

The antimicrobial activity of alfalfa seed extract (50µg, 100µg, 200µg, 300µg /ml) was studied against five bacterial strains (*Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Lactococcus lactis*, *Klebsiella pneumoniae* and *Bacillus cereus*). The maximum inhibition was seen at 300µg/ml concentration of extract. MICs of the extract against *P. aeruginosa* and *S. dysgalactiae* were 0.674 and 1.347 mg/ml respectively, while, MIC of the extract against *E. coli* and *S. aureus* was 2.695 mg/ml [48].

The antifungal activity of the saponin-rich fractions from *Medicago sativa* (aerial parts and roots) was studied against *Candida albicans*. The saponin-rich extracts inhibited *C. albicans* germ tube formation, decreased hyphal growth, reduced yeast adherence and eradicated mature (24 h) *Candida biofilm* [49].

2.7. Dermatological effects

The efficacy of *Medicago sativa* extract (simple cream base containing 7.5 and 15 mg % extract) in the treatment of burn wound induced by NaOH was studied in rat. The extract at both low and high doses significantly increased the level of GSH in burn wound in rat skin tissue compared to the standard drug. A significant decrease of MDA in skin tissues was reported in treatment groups both in low and high dose groups compare to control and standard groups (P<0.01). The concentration of NO was decreased in the skin homogenate of rats treated with both low and high doses of the extracts compare to control group (P<0.01) [50].

The effect of *Medicago sativa* extract on regeneration of pinna cartilage was studied in rabbit. After shaving hairs on ears with depilation cream, the ear were anesthetized by lidocaine 10% and 4 holes were punched with 4 mm diameter in medial situation of each ear. Test ears were treated with the extract of *Medicago sativa* and control ears were treated by normal saline every day. Holes era and the distance of two edges of cartilage were measured daily. Regeneration and healing of the treated holes with extract of *Medicago sativa* was faster than the control (P<0.004). The thickness of cartilage and cell density of chondrocytes and fibroblasts in the newly formed connective tissues in the test ears were more than control [51].

In a clinical trials carried out on volunteers, the application of alfalfa leaves extract cream resulted in human body hair diameter reduction with no side effects. The rate of hair growth was reduced in the three groups treated with 1%, 2% and 5% of Alfalfa cream compared to group which received placebo (P< 0.05). The actual mechanism behind this effect could be attributed to the high concentration of estrogenic components in alfalfa [52].

2.8. Anxiolytic effect

The anxiolytic effects of petroleum ether, chloroform, methanol and aqueous extracts of the aerial parts of *Medicago sativa* was in mice using elevated plus-maze apparatus. Among all extracts, only the methanol extract exhibited significant ($P < 0.05$) anti-anxiety activity by increasing the average time spent, and number of entries in open arms at a dose of 100 mg/kg [17].

The anxiolytic activity of ethyl acetate fraction of the methanolic extract of the seeds of *Medicago sativa* was investigated using elevated plus maze model, hole board test, open field test, stair case test, light/dark exploration, social interaction and locomotor performance in mice. The ethyl acetate of the methanolic extract of the seeds showed significant anxiolytic action at a dose of 100mg/kg, orally [53].

2.9. Neuroprotective effect

The neuroprotective effect of methanol extract of *Medicago sativa* on ischemia and reperfusion-induced cerebral injury was investigated in mice. Pre-treatment with *Medicago sativa* methanolic extract (100 or 200 mg/kg, orally) markedly reduced cerebral infarct size, xanthine oxidase, O_2^- production, thiobarbituric acid-reactive substance, and significantly restored reduced glutathione, superoxide dismutase and total tissue sulfhydryl levels and attenuated impairment in short-term memory and motor coordination. The extract directly scavenged free radicals generated against a stable radical 1,1-diphenyl-2-picrylhydrazyl and O_2^- generated in phenazine methosulphate-nicotinamide adenine dinucleotide systems, and also inhibited XD/XO conversion and resultant O_2^- production [54].

A combined molecular docking and network analysis were carried out to study the mechanisms of the beneficial effect of *Medicago sativa* in neurodegenerative diseases. *Medicago sativa* showed memory improving activities and central nervous protective effects, which attributed to its triterpene saponins contents [55].

2.10. Immunological effects

The effects of alfalfa sprout ethyl acetate extract on disease severity of systemic lupus erythematosus, were studied using autoimmune-prone female MRL-lpr/lpr mice. The onset of proteinuria was delayed, and the life span was significantly longer in the extract treated group compared to the control. Flow cytometric analysis of splenocytes showed a significantly lower percentage of activated T cells in the extract treated group. The *ex-vivo* interferon-gamma and interleukin (IL)-4 production from splenocytes and, TNF- α and IL-1 β production from peritoneal exudate cells were also significantly lower in the extract treated group compared with the control. The extract treated group also had less severe glomerulonephritis [56].

The effects of alfalfa polysaccharides (0, 200, 400 or 800 mg/kg/day bw for 28 days) on immunological and antioxidant functions, as well as its effect on the intestinal morphology were investigated in mice. The results showed that the oral administration of polysaccharides improved the immune functions of mice, significantly (enhanced the white blood cells and lymphocyte counts, and led to improvements in spleen and thymus indices). Alfalfa polysaccharides exhibited significant antioxidant activity by enhancing total antioxidant capacity, superoxide dismutase and glutathione peroxidase activities in heart, kidney and liver, and decreasing the malondialdehyde levels of heart and liver. Administration of alfalfa polysaccharides potently enhanced the small intestinal villous height and the villus-to-crypt ratio, and decreased the crypt depth of duodenum in mice [57].

2.11. Xanthine oxidase inhibitory activity

The leaves crude extract of alfalfa inhibited xanthine oxidase by 51.63% at the concentration of 250 μ g/ml [6].

2.12. Cardioprotective effect

The cardioprotective effect of ethanolic extract of *Medicago sativa* stem was evaluated in isoproterenol induced myocardial infarction in rats. Isoproterenol group showed increased serum levels of liver and cardiac markers and lipid profile with decreased HDL-C level. The pretreatment with the extract reversed the lipid profile level and cardiac and liver enzyme levels to near normal level [58].

2.13. Cytotoxic effect

The cytotoxic effects of *Medicago sativa* extracts were assessed on several sensitive and multidrug-resistant tumor cells lines [mouse leukaemia P388 cell line and its doxorubicin-resistant counterpart (P388/DOX)]. It appeared that the growth inhibitory effect of alfalfa leaf extracts was mediated through the induction of apoptosis, as evidenced by DNA fragmentation analysis. The execution of programmed cell death was achieved via the activation of caspase-3, leading

to PARP cleavage. Fractionation of toluene extract (the most active extract), led to the identification of 3 terpene derivatives and 5 flavonoids. Among them, (-)-medicarpin, (-)-melilotocarpin E, millepurpan, tricin, and chrysoeriol showed cytotoxic effects on P388 and P388/DOX cells [26].

2.14. Hepatoprotective effect

The effect of lyophilized aqueous extract of *Medicago sativa* against CCl₄ - induced oxidative stress and liver injury was studied in rats. Pretreatment with alfalfa for three weeks prior to the administration of CCl₄ significantly prevented the increase in the serum levels of hepatic marker, LDL, VLDL levels and reduced oxidative stress indicated by elevated non-protein sulfhydryl and total protein concentration. The histopathological examination of the livers also showed that the alfalfa extract reduced the incidence of liver lesions induced by CCl₄ [59].

2.15. For the treatment of metabolic syndrome

Medicago sativa grown under high salinity conditions was added to experimental diets and assayed in an experimental model of spontaneously hypertensive rat. It caused a slight decrease on blood pressure, a reduction in the risk of kidney stone formation, a protective action against oxidative damage in fatty liver disease, as well as an improvement of glucose metabolism. Alfalfa was an efficient functional food for the dietary prevention and treatment of several metabolic alterations, associated with metabolic syndrome [60].

2.16. In the treatment of anemia

A randomized controlled two-arm trial was carried out on 102 anemic adolescent girls aged 14–18 years, used leaf concentrate over 3 months as an alternative to iron and folic acid supplements. At the end of the trial, none of the eighty-six remaining girls were severely anemic, nine (10.5%) were moderately anemic and twenty-six (30.2%) were mildly anemic, and fifty-one (59.3%) had normal Hb levels (12 g/dl). The results revealed that the leaf concentrate is effective, and more palatable, alternative to Fe and folic acid supplements for treating anemia in adolescent girls [30].

2.17. Anti scorbutic activity

The anti scorbutic activity was attributed to high Vitamin C content. It can be used to manage scurvy by its incorporation in the diet [61].

3. Toxicity, and side effects

The effect of hydroalcoholic extract of *Medicago sativa* on liver function, blood biochemical factors and coagulation system parameters was studied in male rats. The results showed that the use of the extract decreased significantly the serum levels of ALP, ALT, and glucose concentration in the experimental groups, compared to the control group. Furthermore, alfalfa increased total protein and fibrinogen in a dose dependent manner in the experimental groups (P <0.01 and P <0.001 respectively) [62].

Moderate consumption of *Medicago sativa* leaves in teas and capsules is generally considered safe and without significant side effects. Aggravation of lupus, or promotion of lupus-like symptoms have been reported from the ingestion of large amounts of *Medicago sativa* seeds and sprouts, an action attributed to the amino acid, canavanine [63].

No human or animal studies on *Medicago sativa* in pregnancy or lactation, but herbalists considered *Medicago sativa* to be safe during pregnancy because farmers do not restrict livestock from feeding on *Medicago sativa* during pregnancy or lactation. *Medicago sativa* feed increased milk yield, lowered fat, and increased milk protein in dairy cows [64].

4. Conclusion

This review discuss the chemical constituent, pharmacological and therapeutic effects of *Medicago sativa* as promising herbal drug because of its safety and effectiveness.

Compliance with ethical standards

Acknowledgments

We acknowledged the dean of Thi qar college of medicine, the dean of Thi qar college of pharmacy and the dean of Faculty of veterinary medicines for the scientific support.

Disclosure of conflict of interest

The authors confirm that this paper's content has no conflict of interests.

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