WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 9, 683-700. Review Article ISSN 2277– 7105

A SYSTEMATIC REVIEW ON ONE OF THE NUTRACEUTICAL POTENTIAL PLANT *MEDICAGO SATIVA* (ALFALFA)

Megha S. Shah[1]*, Dhumal Supriya[2], Ghavate Mayuri[3] and Dr. Rajesh J. Oswal[4]

Department of Pharmacognosy, Genba Sopanrao Moze college of Pharmacy, Wagholi, Pune-412207, India.

*[1]Assistant Professor Department of Pharmacognosy, Genba Sopanrao Moze College of Pharmacy, Wagholi, Pune.

[2,3]UG Student, Genba Sopanrao Moze College of Pharmacy, Wagholi, Pune.

[4]Principal, Genba Sopanrao Moze College of Pharmacy, Wagholi, Pune.

Article Received on
01 July 2020,
Revised on 21 July 2020,
Accepted on 11 August 2020,
DOI: 10.20959/wjpr20209-18453

***Corresponding Author**
Megha S. Shah
Assistant Professor
Department of Pharmacognosy, Genba Sopanrao Moze College of Pharmacy, Wagholi, Pune.

ABSTRACT

The role of alfalfa (*Medicago sativa* L.) in the development of agricultural production and intensification of forage production is due to the high potential of biomass on the one hand anti-oxidative and nutritional value on the other hand. The ever-increasing growth in the size of the human population is causing a growing demand for animal protein. This demand has spurred an interest in cheaper, plant-derived proteins that have an equally rich protein composition as alternatives to proteins of animal origin. Lucerne *Medicago* (L.) is the most popular and widespread protein-yielding crop which is grown in cool-climate regions. Extracts and concentrates of lucerne contain, along with proteins, also many vitamins, nutritive substances and secondary metabolites. Due to valuable chemical constituents which show phytobiotic action on humans. Lucerne is used in folk medicine and phytotherapy. *Medicago sativa* seems to hold great potential for in-depth investigation for various biological activities, especially their effects on central nervous and cardiovascular system. Through this review, the authors hope to attract the attention of natural product researchers throughout the world to focus on the unexplored potential of *M. sativa*, and it may be useful in developing new formulations with more therapeutic value.

KEYWORDS: *Medicago sativa* L., phytotherapy, Lucerne, Lipid lowering activity, Systemic lupus erythematosus.

INTRODUCTION

The size of the world's human population has continually increased with a concomitant growth in the demand for animal protein and other protein-based foodstuffs. The production of animal protein relies on the development of animal farming and on an increased demand for animal feed, which severely taxes the available resources of land, capital and energy required to grow fodder plants, hurts the environment (e.g., by animal droppings, the emission of nitrogen and greenhouse gases and the warming of the climate) and compromises biodiversity. These are the reasons behind an on-going search for new, cheaper, safer and more environmentally friendly protein sources and for other valuable food additives and secondary metabolites of plant origin.[1]

Food shortages and the resulting undernutrition are very serious issues in developing countries both in terms of the wellbeing of local communities and of their economic development. An undernourished population is often in poor health and prone to different disorders and unable to profit from education and to perform well at their jobs.[2] Prolonged undernutrition seriously affects the physical development and the nervous systems of the affected youths. Therefore, in many African, South American countries, in India and other Asian countries, and even in Europe, possibilities are being explored for the production of plant proteins which have phytobiotic activity and which could be extracted from locally grown crops well suited to the local habitat. Furthermore, approximately 5% of the total human population do not tolerate gluten, a protein which is present in the grains of rye, barley and oats and is the predominant protein in wheat grain. Such people are inclined to give up gluten-containing food altogether[3] and to use alternative protein sources.

Medicago sativa Linn., commonly known as the "father of all foods" (al-fal-fa), is a perennial herbaceous leguminous plant species that originated in Asia. In Poland, farmers call lucerne "the queen of fodder crops" because of its high nutritional value. This is the most ancient plant, cultivated throughout the world as a fodder plant. In America, *M. sativa* has been extensively cultivated since the arrival of Europeans. *M. sativa* has been grown for a variety of purposes such as soil improvement, animal feed and medicinal uses.[4]

Alfalfa, also called **lucerne** and called ***Medicago sativa*** in binomial nomenclature, is a perennial flowering plant in the legume family Fabaceae. It is cultivated as an important forage crop in many countries around the world. It is used for grazing, hay and silage, as well as a green manure and cover crop. The name alfalfa is used in North

America. The name lucerne is the more commonly used name in the United Kingdom, South Africa, Australia and New Zealand.[5]

Scientific classifications[5]

Kingdom: Plantae;

Division: Magnoliophyta;

Class: Magnoliopsida;

Order: Fabales;

Family: Fabaceae;

Subfamily: Faboideae;

Tribe: Trifolieae;

Genus: *Medicago*;

Species: *sativa*;

Binomial name: *M. sativa* Linn.

Figure 1: Plant of alfalfa, seed and its sprouts.

GEOGRAPHICAL DISTRIBUTION

Iran formerly Persia is regarded as the area in which the genus *Medicago* (L.) originated and from which it rapidly spread to all continents. Greece was the first European country to which lucerne arrived from ancient Persia (Media). The ancient Greeks called the plant "the medic grass" and the Latin version of the name "herba medica" gave rise to "*Medicago*", the name under which Linnaeus introduced lucerne into the botanical lexicon. In antiquity, lucerne was the principal fodder for horses and it spread along the caravan routes (as well with cavalry and chariot horses of invading armies) of the Persians, Greeks and Romans. It was carried as far a field as China, India, Greece, North Africa and Spain. In the beginning of the 16th century lucerne cultivation began in France and, by the 18th century, it reached Russia, Austria, Germany and Switzerland. Lucerne is grown between the 30 and 60th parallels of the northern and southern hemisphere and the area covers eastern Asia, the Middle East, Europe, the northern part of Africa, the USA, the central and southern part of Canada, northern Mexico, the central part of South America, Australia and new Zealand.

At present, lucerne *Medicago* (L.) is the world's major leguminous fodder. In 2009, it was grown in an area of 30 million hectares all across the world, and 11.9 million hectares was grown in North America, 7 million hectares in South America, 7.12 million hectares in Europe, 2.23 million hectares in Asia, and 1.75 million hectares in Africa and Oceania.[6] Lucerne is a very valuable small-seed legume that can produce an annual crop of 45-90 tone/hectare of fresh herb which, when dried, yields 15-22 tone/hectare of protein-rich fodder.[7] The protein and crude fibre content of the crop is closely related to its developmental stage at harvest.

Habitat

Lucerne is native to warmer temperate regions. It has been grown as a fodder crop for animals since the ancient Greek and Roman times. At present, perennial and multi-harvest lucernes are of major importance in Europe because they have a deep root system capable of extracting water and nutrients from the deep layers of the soil. The deep root system also makes Lucerne resistant to drought. Lucerne should be grown in a fertile soil at a pH close to neutral. Similar to other legumes, lucerne roots are colonized by bacteria that fix nitrogen from the air. The nitrogen in the vegetable residues is retained in the soil, thereby enriching the soil environment or it is transferred to other non-legume crops. Lucerne is harvested three, four or sometimes as many as five to six times a year. Juvenile intensively mowed plants have the highest nutritional value. Apart from proteins, minerals and vitamins, lucerne also contains secondary metabolites which have phytobiotic activity in humans and animals.[1]

MORPHOLOGY

The mature *M. sativa* plant is characterized by a strong **taproot**. This taproot may eventually surpass 6 m or more in length with several to many lateral roots connected at the crown when *M. sativa* is grown in deep, well drained, moist soils.

The crown, a complex structure near the soil surface, has perennial meristem activity, producing buds that develop into **stems**.

Tri- or multi-foliolate **leaves** form alternately on the stem, and secondary and tertiary stems can develop from leaf axils. A plant in a typical forage production field has between 5 and 15 stems and can reach nearly 1 m in height.

Flowers vary in color yet purple, variegated, yellow, cream and white are the most common. After pollination, these flowers most commonly produce spiral-shaped **seed** pods.[8]

CHEMICAL CONSTITUENTS

M. sativa has been reported to contain a variety of phytochemicals. It has following different classes of phytoconstituents.

Alkaloids: asparagines, trigoneline, stachydrine, l-homostachydrine.

Amino acids: medicanine, lysine, arginine, histidine, tyrosine, phenylalanine, methionine, aspartic acid, glutamic acid, asparagine, serine, alanine, threonine.

Carotene

Coumarins: myrsellinol, scopoletin, esculetin, 4-coumaric acid.

Digestive enzymes

Enzymes in Leaves: isoflavone reductase, vestitone reductase, iminopeptidase and two aminopeptidases.

Minerals: K, Mg, Zn, Cu, Al, B, Cr, Co, Mn, Mo, Se, Si, Na, Sn. {Ca, P, Fe from leaves}

Non-protein amino acids: l-canaverin.

Organic acids: citrate, malate, malonate, succinate, fumarate, lactate, benzoate.

Phenolic compounds: *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acids, salicylic acid, sinapic acids, caffeic acid, hesperetin, naringenin, chlorogenic acid, tannic acid, heterosides.

Phytoestrogens: coumestrol, genistein, formometin, diadzein, biocanine A.

Phytosterols: β-sitosterol, stigmasterol.

Polyamines: norspermidine, norspermine.

Protein: ferritin from seed, protein phosphatase 2A holoenzyme, β-amylase from root

Saponins: soyasapogenols, hederagenin, medicagenic acid.

Vitamins from leaves A, B_1, B_6, B_{12}, C, D, E, K, niacin, pantothenic acid, biotin, folic acid

Volatile components from complex flower: Terpenes, limonene, linalool, *trans*-ocimene, furanoids, nonadienal, 2-methyl 4-pentenai, benzaldehyde, ethyl benzaldehyde, alcohols, butanol, hexanol, octanol, pentan-3-ol, 3 methylbutanol, *trans*2-pentenol, *trans*-2-hexenol, *trans*-3-hexenol, pent-1- en-3-ol, ott-I-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-pentenal, *trans*-2-hexenal, *trans*-2- nonenal, *trans*-2,4-hexadienal, furane-2-ethyl.[4]

Flavonoid glycosides constitute important group of plant secondary metabolites **aerial parts** of alfalfa. This class of natural products play significant role in different physiological processes. It was shown that flavonoids of alfalfa are glycosides of four flavone aglycones: apigenin, luteolin, tricin and chrysoeriol. All flavonoid glycosides possesed glucuronid acid in sugar chain. Some of them were acylated with ferulic, coumaric or sinapic acids. It was shown that dominant flavonoids of alfalfa were the flavones: tricin and apigenin glycosides (65–72% of total). The concentration of luteolin and chrysoeriol glycosides did not exceed 30% of the total.[9]

Nine flavones and adenosine have been identified in **aerial parts of alfalfa** and their structures were established by spectral (FABMS and NMR) techniques. Five of the identified compounds, including apigenin 7-O-[beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranosyl]-4'-O-beta-D-glucuronopyranoside, apigenin 7-O-[2-O-feruloyl-beta-D-glucuronopyranosyl (1-->2)-O-beta-D-glucuronopyranosyl]-4'-O-beta-D-glucuronopyranoside, apigenin 7-O-[2-O-feruloyl-[beta-D-glucuronopyranosyl(1-->3)]-O-beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranoside], apigenin 7-O-[2-O-p-coumaroyl-[beta-D-glucuronopyranosyl(1-->3)]-O-beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranoside], and luteolin 7-O-[2-O-feruloyl-beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranosyl]-4'-O-beta-D-glucuronopyranoside, have not been reported before in the plant kingdom. Additionally, five known compounds, including apigenin 7-O-beta-D-glucuronopyranoside, apigenin 4'-O-beta-D-glucuronopyranoside, apigenin 7-O-[beta-D- glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranoside], luteolin 7-O-beta-D-glucuronopyranoside and adenosine, were identified.[10]

Three flavones, including 4'-O-[2'-O-E-feruloyl-O-beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranoside] apigenin, 7-O-beta-D-glucuronopyranosyl-4'-O-[2'-O-E-feruloyl-O-beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranoside] apigenin and 7-O-beta-D-glucuronopyranosyl-4'-O-[2'-O-p-E-coumaroyl-O-beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucuronopyranoside] apigenin have been identified in alfalfa variety Artal. The known flavone 7-O-[2-O-E-feruloyl-[beta-D-glucuronopyranosyl(1-->3)]-O-beta-D-glucuronopyranosyl(1-->2)-O-beta-D-glucurono-pyranoside] apigenin was also isolated. The structures of these compounds were deduced on the basis of their spectral data.[11]

Table 1: Different constituent with pharmacological action.[6]

Chemical constituent	Obtained from part of plant	Pharmaological effect	Mechanism of action	Side effect
Amino acids	Whole plant concentrate	Essential and semi-essential amino acids.	Good nutritional value	
L-canavanine	Larger quantities in the leaves than it does in the shoots, highest amounts have been identified in the seeds and sprouts	Potent anti-cancer		If ingested in a large amount shows cytotoxic and antimetabolic activity which disturbs DNA and RNA synthesis. It may also cause auto-immune disorders in humans.
				Exacerbated the development of systemic lupus erythaematosus (SLE) in monkeys
				L-canavanine-containing lucerne sprouts induced auto-immune haemolytic Anaemia
Saponins (water soluble, soap-like foaming glycosides)	Much larger quantities in the roots of lucerne than they do in the aerial parts	Anti-arteriosclerotical activity	By inhibiting the deposition of cholesterol in the blood system in monkey.	Haemolysis (the result was that haemoglobin, which is otherwise present in erythrocytes, permeated into the blood serum) Haemolysis may result from an
			It reduced concentration of cholesterol in the aortic sudanophilia, in the aortic infima-plus-media and in the liver of rats	

			In the human body, saponins form insoluble complexes with cholesterol which are subsequently excreted in the faeces.	ecogenetic disorder known as favism, a condition related to the deficiency of glucose-6-phosphate dehydrogenase which is crucial for the carbohydrate metabolism of erythrocytes and leads to the production of glutathione, a strongly oxidizing enzyme that protects erythrocytes against disintegration. In a damaged digestive tract, saponins may penetrate into the bloodstream and cause haemolysis.
Saponin		Antibacterial, antifungal and antiviral properties	Restrict the development of protozoa, control the gram-positive bacteria *Bacillus cereus*, *B. subtilis*, *Staphylcoccus ureus* and *Enterococcus faecalis* and some yeast-like fungi	Negatively affect the reproductive potential of humans and animals
Saponins	Aerial parts and roots	Reduction of hyphal growth, yeast adhesion and biofilm formation both in humans and in animals		
Phenolic compound	Water extract of seed	Postprandial glycaemia in individuals with type 1 and type 2 diabetes	High antioxidant activity lucerne sprouts have been reported to show a nutraceutical effects in humans	
Flavonoids, phytoestrogens, and ascorbic acid	Lucerne extract	Inhibiting the formation of cholesterol, protecting people against arteriosclerosis and preventing the development of coronary heart		

		disease in postmenopausal women		
Phytoestrogens, (isoflavones) and coumestrol.		Oestrogenic activity similar to that of the female hormones responsible for the development of the female reproductive organs	Phytoestrogens mitigate menopause-related ailments in women, help keep osteoporosis in check, and restrain the development of hormone-dependent cancers of the breast and prostate gland in men	
Proteins, vitamins and mineral	Extracted from the above-ground parts of various plants	Person suffering from health problems related to malnutrition or to the absence of some nutrients from their diet.		

PHARMACOLOGICAL EFFECT

Antiatheroscerotic activity

A semipurified diet containing 1.2 mg of cholesterol/Cal was fed to cynomolgus monkeys (*Macaca fascicularis*). At the end of 6 months, a group of 18 animals was killed for evaluation of atherosclerosis in the aorta and the coronary arteries. The remaining monkeys were assigned to three groups of 18 animals each and fed, during the following 18 months, semipurified diets containing 0.34 mg of cholesterol/Cal with or without alfalfa meal, or a diet consisting entirely of Monkey Chow. A decrease in cholesterolemia and plasma phospholipid levels, normalization in the distribution of plasma lipoproteins, and reduction in the extent of aortic and coronary atherosclerosis were observed in monkeys fed the semipurified diet containing alfalfa, although the intake of cholesterol remained as high as in the usual American diet. These changes, also observed in monkeys fed a chow diet almost devoid of cholesterol, suggest that alfalfa counteracts the atherogenic effect of dietary cholesterol.[12,13]

Lipid lowering effect

Partial ileal bypass (PIB) and ingestion of alfalfa are both known to lower plasma cholesterol (C) levels. During an 18-week period, the combined effects of both were studied in four randomized groups of rabbits receiving C-free, hypercholesterolemia-inducing, semisynthetic diets 3 weeks after sham or PIB surgery. The diets, with or without alfalfa, had similar overall compositions of fat, protein, carbohydrate and fiber. We measured blood C, triglycerides (T), and lipoprotein fractions of both C and T at biweekly intervals. In vivo liver and small-bowel synthesis of C, fatty acids (FA), and nonsaponifiable lipids (NSL) were determined with radioactive ^{14}C-acetate at the end of the study. The results were evaluated by means of analysis of variance using unweighted cell means. The combined PIB and alfalfa modalities significantly lower C levels in serum, plasma, low-density lipoproteins and high-density lipoproteins by 66%, 71%, 85% and 35%, respectively. However, due to alfalfa, a significant increase of 49% was observed in plasma T when both treatments were combined. Liver FA synthesis was significantly decreased (65%) with PIB and increased (161%) with alfalfa; when the two treatments are combined, a non significant response was observed. Similarly, this inverse relationship for PIB and alfalfa was seen for C and NSL synthesis. Small-bowel FA synthesis was significantly decreased (72%) by the combination of PIB and alfalfa. We conclude that alfalfa suppresses, in part, the physiologic rebound effect of PIB surgery by increasing hepatic C and NSL synthesis; inversely, PIB surgery inhibits the additive effect in the liver synthesis of FA produced by alfalfa. Alfalfa and PIB alone, and synergistically, decrease total small-bowel lipid synthesis, specifically that of FA. Alfalfa is an effective adjuvant to PIB for reducing total and lipoprotein C fractions.[14]

Prevention of Hypercholesterolaemia

Experiments are described which were designed to find out how alfalfa prevents hypercholesterolaemia in rabbits. It was found that rabbits with shortened small intestine (ileal bypass) required less alfalfa to prevent blood serum cholesterol elevation than rabbits with normal length of gut and that rabbits with ileal bypass absorbed less cholesterol than normal rabbits. It was found that rabbits receiving 600 mg. of cholesterol daily required more alfalfa than those receiving 300 mg. to prevent hypercholesterolaemia. The lower amount of alfalfa required to prevent a rise in the blood serum cholesterol in rabbits with shortened small intestine could be due to decreased ability to absorb cholesterol from the intestinal lumen. The observation that more alfalfa was required to prevent a blood serum cholesterol rise when rabbits received higher doses of cholesterol tends to support the hypothesis that alfalfa prevents hypercholesterolaemia by forming unabsorbable complexes with cholesterol in the intestinal lumen.[15]

The results given above together with previous finding, that alfalfa prevents the absorption of cholesterol by the gut, allow certain deductions to be made as to exactly how alfalfa prevents hypercholesterolaemia. Three mechanisms of action may be suggested: 1. The effect may be a bulk effect of undigested fibres. 2. Some component of alfalfa may be forming inabsorbable complexes with cholesterol in the gut. 3. Some component of alfalfa may be blocking the absorptive mechanism for cholesterol in the intestinal mucosa.[16]

Effect of saponin on Cholesterol Intestinal absorption

Five to 20 mg of saponins obtained from alfalfa tops or roots were introduced intragastrically in rats also receiving oral and intravenous ring-labeled cholesterol. The saponins were tested before and after partial acid hydrolysis. Absorption of cholesterol was determined by estimation of fecal sterols and by a dual isotope technique involving assay of plasma radioactivity. Alfalfa top saponins (nonhydrolyzed) reduced absorption of cholesterol. Acid hydrolysis of alfalfa top or root saponins enhanced their ability to inhibit cholesterol absorption.[17]

Alfalfa root saponins prevented the expected increase in plasma cholesterol associated with the Ingestion of a semipurified high-butter, high-cholesterol diet in monkeys. Experiments in rats indicate that alfalfa root saponins decrease cholesterol intestinal absorption.[18]

Intestinal absorption of cholesterol was measured in control rats fed semipurified diets and in rats fed alfalfa meal, in which saponins had been previously extracted, or this extracted material plus alfalfa saponins. A dose of 2 mg radioactive cholesterol was administered intragastrically, and fecal excretion of labeled neutral steroids measured. Absorption of cholesterol was about 76% in control animals, and about 47% in alfalfa-red rats. Extraction of saponins from **leaves and stem** of alfalfa eliminated the cholesterol absorption-lowering effect, while addition of 0.26% alfalfa saponins to the extracted alfalfa restored its activity. The results demonstrate that alfalfa saponins are responsible for the effect of alfalfa meal in reducing cholesterol absorption, and that alfalfa fiber is not involved in this activity.[19]

The effects of alfalfa top **saponins on cholesterol and bile acid balance** in eight *cynomolgus macaques* (*Macaca fascicularis*). The monkeys ate semipurified food containing cholesterol with or without added saponins. The saponins decreased cholesterolemia without changing the levels of high density lipoprotein-cholesterol; hence, they reduced the total cholesterol/high density lipoprotein-cholesterol ratio. Furthermore, they decreased intestinal absorption of cholesterol, increased fecal excretion of endogenous and exogenous neutral steroids and bile acids, and decreased the percent distribution of fecal deoxycholic and lithocholic acids. The fecal excretion of fat was also slightly increased, but steatorrhea did not occur.[20]

Antihyperlipidemic effect

The influence of alfalfa meal on hyperlipidemia induced by dietary cholesterol was examined and changes in serum total cholesterol (TC), triglycerides (TG) and non-esterified fatty acid (NEFA) in rabbits were recorded. Serum lipid levels of groups treated with alfalfa meal (R-1) as well as those not treated (R-2) were found to be elevated. TC of R-1 was lower than that of R-2. The inhibition effect of alfalfa meal on elevation of TC was apparent to a certain extent. The inhibition effect of alfalfa meal on elevation of TG and NEFA was greater than that of TC, thus it is suggested that alfalfa meal can be successfully utilized for experiments with lipids, when the rabbit is the experimental animal of choice.[21]

A study and showed that highest saponin content extract just before fruiting stage (free from both coumestrol and canavanine) of *M. sativa* exhibited significant **hypocholesterolemic and antiatherosclerotic** activity. This study proved that *M. sativa* was found to safely reduce natural cholesterol and to possess a strong anti-atherosclerotic activity. The extracts produced the most significant decrease in total cholesterol and LDL-cholesterol by 85.1 and 88%, respectively, of the corresponding levels in hypercholesterolemic rabbits. This decrease was more significant than that produced by gemfibrozil (73 and 74%) upon concomitant administration with a cholesterol enriched diet using the same animal model at the tested dose level.[22]

Antifungal and hemolytic activity

Aerial parts of alfalfa contains a relatively high Level of saponins which are medicagenic acid and/or hederagenin glycosides, which have mostly or entirely soyasapogenol glycosides. which appear to be promising sources for obtaining antifungal saponins for commercial development. It also shows hemolytic activity.[23]

Anti diabetic activity

Medicago sutiva (lucerne) is used as a traditional plant treatment of diabetes. In the present study, administration of lucerne in the diet and drinking water reduced the hyperglycaemia of streptozotocin-diabetic mice. An **aqueous extract of lucerne seed** stimulated 2-deoxy-glucose transport, glucose oxidation and incorporation of glucose into glycogen in mouse abdominal muscle. The effect of extract was potentiated by 16.7 mhl-glucose and by 1 mM-3-isobutyl-1-methylxanthine. L-Alanine (10 mM) and a depolarizing concentration of KCI (25 m) did not augment the insulin-releasing activity of lucerne. Activity of the extract was found to be heat stable and largely acetone insoluble, and was enhanced by exposure to acid and alkali (0.1 M-HCI and NaOH) but decreased 25% with dialysis to remove components with molecular mass < 2000 Da. The results demonstrate the presence of antihyperglycaemic, insulin-releasing and insulin-like activity in the traditional antidiabetic plant, *Medicago sutiva.*[24,25]

Act as Antimycotic Agent

Compound G2, 2-β-hydroxy-3-β-o-(β-D-glucopyranosyl)-Δ^{12}-oleanene-23, 28-dionic acid, isolated from alfalfa roots, demonstrated considerable activity against *Cryptococcus neoformans*. Compound G2 exhibited rapid killing of this fungus suggesting that it might be a useful active agent in the treatment of cryptococcosis.[26]

The mode of action of the antimycotic alfalfa root saponin, medicagenic acid 3-O-beta-D-glucopyranoside (compound G2), which possesses a pronounced antifungal activity against medically important yeasts and dermatophytes, was studied in *Saccharomyces cerevisiae*. Compound G2 caused lethal leakage of ions out of the yeast cells. Exposure of *S. cerevisiae* to compound G2 resulted in a disappearance of the main sterol, ergosterol, from the cell membranes, suggesting that compound G2 was highly specific for ergosterol. Independently, chemical data indicated that compound G2 forms stable complexes with both ergosterol and cholesterol. Addition of cholesterol or ergosterol protected the cells of *S. cerevisiae* and several pathogenic yeasts from the inhibitory activity of compound G2 by producing a higher ratio of sterols (mainly ergosterol) to phospholipids in the membranes. The fact that an amphotericin B-resistant *Candida tropicalis* was susceptible to G2 suggested that its mode of action was different from that described for polyene antibiotics. This was also confirmed by the finding that 0.2 M KCl did not protect *S. cerevisiae* cells against ion leakage with G2, but did so with amphotericin B.[27]

Estrogenic effect

Methanol extract of *M. sativa* has been shown significant estrogenic activity using an estrogen-dependent MCF-7 breast cancer cell proliferation assay. The extract showed significant competitive binding to estrogen receptor β (ER). The pure estrogen antagonist, ICI 182,780, suppressed cell proliferation induced by the extract, suggesting an ER-related signaling pathway was involved. The ER subtype-selective activities of extract were examined using transiently transfected human embryonic kidney (HEK 293) cells. Methanol extract exhibited preferential agonist activity toward ER. Phytoestrogens of the extract were determined to be responsible for estrogenic activity.[28]

Treatment of neurovegetative menopausal

Extract of the **leaves** from *M. sativa* has been shown to be used in treatment of neurovegetative menopausal symptoms in women. Hot flushes and night sweating completely disappeared with the treatment of *M. sativa* extract. The plant product induced a significant increase in prolactin and thyroid stimulating hormone response to thyroid releasing hormone. Basal levels of estradiol, luteinizing hormone, follicle stimulating hormone, prolactin, and thyroid stimulating hormone were unchanged. Thus, *M. sativa* suggested having a central slight antidopaminergic action without side effects.[29]

TOXICITY EFFECT

Systemic lupus erythematosus (SLE) has been observed in monkeys fed alfalfa seeds. proposed hypothesis for this syndrome may be triggered by L-canavanine found in alfalfa seeds. L-Canavanine, $H_2N\text{-}C(=NH)\text{-}NH\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)COOH$, is the guanidinoxy structural analog of arginine; it constitutes about 1.5 percent of the dry weight of **alfalfa seeds** and alfalfa **sprouts** and is toxic to many organisms, including mammals may be involved in the pathogenesis of the SLE-like syndrome since this amino acid induced certain hematologic and serologic abnormalities characteristic of the syndrome.[30]

DISCUSSION

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. The present review emphasizes the phytochemical, pharmacological and clinical reports on *M. sativa*. Saponins, flavonoids, phytoestrogens, coumarins, alkaloids, amino acids, phytosterols, vitamins, digestive enzymes and terpenes constitute major classes of phytoconstituents of the plant. *M. sativa* has been used since centuries as homoeopathic and Ayurvedic medicine for a variety of ailments.

The plant is widely used in foods and is listed by the Council of Europe as a source of natural food flavor (category N2 and N3). These categories indicate that *M. sativa* can be added to foodstuffs in small quantities with a limitation on the concentrations of an active ingredient in the final product. In the USA, *M. sativa* is listed as GRAS (generally recognized as safe).

The presence of a wide range of chemical compounds indicates that the plant could lead the way for the development of novel agents having good biological activity. Exploration of the chemical compounds of the plant will provide the basis for developing such a lead. Many chemical compounds are present in the plant but isolation of active constituents by using various appropriate chromatographic techniques should be carried out. Further studies can be carried out using different extraction methods such as microwave extraction, isolation and identification of active compounds, pharmacological screening of extracts as well as isolated compound(s) so that accuracy of results can be obtained and use of extracts can be made in different herbal formulation. It is necessary to exploit its maximum potential in the field of medicinal and pharmaceutical sciences for novel and fruitful application.

REFERENCES

1. Aiking H. Future protein supply. Trends in Food Science & Technology, 2011; 22: 112-120.
2. Davys MNG, Richardier F-C, Kennedy D, de Mathan O, Collin SM, Subtil J, Bertin E, Davys MJ. Leaf Concentrate and Other Benefits of Leaf Fractionation. In: Thompson, B., and Amoroso, L. (eds) Combating Micronutrient Deficiencies: Food-based Approaches. Rome, Italy: CAB International and FAO, 2011; 338 365.
3. Podolska G, Konopka I, Dziuba J. Response of grain yield, yield components and allergic protein content of winter wheat to drought stress. Proceedings of the 9th European Society of Agronomy Congress (ESA), 2006; 4-11: 473-474.
4. Kundan Singh Bora and Anupam Sharma. Phytochemical and pharmacological potential of *Medicago sativa*: A review. Pharmaceutical Biology, 2011; 49(2): 211–220.
5. Heywood VH, Ball PW. Leguminosae. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, Eds. Flora Europaea, Vol II. Cambridge: Cambridge University Press, 1968: 245–260.

6. Eliza Gaweł, Mieczysław Grzelak, Magdalena Janyszek. Lucerne (*Medicago sativa* L.) in the human diet – Case reports and short reports. Journal of Herbal medicine, 2017; S2210-8033(17)30052-0
7. Gaweł E, Żurek J. Nutritional Value of selected lucerne cultivars. Biuletyn IHAR, 2003; 225: 167-174.
8. Teuber LR, Brick MA. Morphology and anatomy. In: Hanson AA, Barnes DK, Hill Jr RR, eds. Alfalfa and Alfalfa Improvement. Madison, WI, 1988: 125–162.
9. Sylwia go£awska, iwona £ukasik, ireneusz kapusta and bogdan janda. Analysis of flavonoids content in alfalfa. Ecological chemistry and engineering, 2010; 17(2–3): 261-267.
10. Stochmal A, Piacente S, Pizza C, Riccardis FD, Leitz R, Oleszek W. Alfalfa (*Medicago sativa* L.) flavonoids. Apigenin and luteolin glycosides from aerial parts. Journal of Agricultural and Food Chemistry, 2001; 49(2): 753-8.
11. Stochmal A, Simonet AM, Macias FA, Oliveira MA, Abreu JM, Nash R, Oleszek W. Acylated apigenin glycosides from alfalfa (*Medicago sativa* L.) var. Artal. Phytochemistry, 2001; 57(8): 1223-6.
12. Malinow MR, McLaughlin P, Naito HK, Lewis LA, McNulty WP. Effect of alfalfa meal on shrinkage (regression) of atherosclerotic plaques during cholesterol feeding in monkeys. Atherosclerosis, 1978; 30(1): 27-43.
13. Srinivasan SR, Patton D, Radhakrishnamurthy B, Foster TA, Malinow MR, McLaughlin P, Berenson GS. Lipid changes in atherosclerotic aortas of *Macaca fascicularis* after various regression regimens. Atherosclerosis, 1980; 37(4): 591-601.
14. Esper E, Barichello AW, Chan EK, Matts JP and Buchwald H. Synergistic lipid-lowering effects of alfalfa meal as an adjuvant to the partial ileal bypass operation. Surgery, 1987; 102(1): 39-51.
15. Barichello AW and Fedoroff S. Effect of Ileal Bypass and Alfalfa on Hypercholesterolaemia. British Journal of Experimental Pathology, 1971; 52(1): 81–87.
16. Cookson FB and Fedoroff S. Quantitative relationships between administered cholesterol and alfalfa required to prevent hypercholesterolaemia in rabbits. British Journal of Experimental Pathology, 1968; 49(4): 348-355.
17. Malinow MR, McLaughlin P, Papworth L, Stafford C, Kohler GO, Livingston AL, Cheeke PR. Effect of alfalfa saponins on intestinal cholesterol absorption in rats. The American Journal of Clinical Nutrition, 1977; 30(12): 2061–2067.

18. Mallnow MR, McLaughlin P, Kohler GO, Livingston AL. Prevention of elevated cholesterolemia in monkeys by alfalfa saponins. Steroids, 1977; 29(1): 105-110.
19. Malinow MR, McLaughlin P, Stafford C, Livingston AL, Kohler GO, Cheeke PR. Comparative effects of alfalfa saponins and alfalfa fiber on cholesterol absorption in rats. American Journal of Clinical Nutrition, 1979; 32(9): 1810-2.
20. Malinow MR, Connor WE, McLaughlin P, Stafford C, Lin DS, Livingston AL, Kohler GO and McNulty WP. Cholesterol and bile acid balance in *Macaca fascicularis*. Effects of alfalfa saponins. Journal of Clinical Investment, 1981; 67(1): 156-162.
21. Yanaura S and Sakamoto M. Effect of alfalfa meal on experimental hyperlipidemia. Nippon Yakurigaku Zasshi, 1975; 71(5): 387-393.
22. Khaleel AEG, Mohamed Z, El-Maraghy SA, Hifnawy MS, Abdel-Sattar E. Study of hypocholesterolemic and antiatherosclerotic properties of *Medicago sativa* L. cultivated in Egypt. Yaowu Shipin Fenxi, 2005; 13: 212–218
23. Jurzysta M and Waller GR Antifungal and hemolytic activity of aerial parts of alfalfa (Medicago) species in relation to saponin composition. Advance in Experimental Medicine and Biology, 1996; 404: 565-574.
24. Alison MG and Peter RF. Pancreatic and Extra-Pancreatic Effects of The Traditional Anti-Diabetic Plant, *Medicago Sativa* (Lucerne). British Journal of Nutrition, 1997; 78: 325-334.
25. Swanston-Flatt SK, Day C, Bailey CJ and Flat PR. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia, 1990; 33: 462-464.
26. Polacheck I, Zehavi U, Naim M, Levy M and Evron R. The susceptibility of *Cryptococcus neoformans* to an antimycotic agent (G2) from alfalfa. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, 1986; 261(4): 481-486.
27. Polacheck I, Levy M, Guizie M, Zehavi U, Naim M and Evron R. Mode of action of the antimycotic agent G2 isolated from alfalfa roots. Zentralbl Bakteriol, 1991; 275(4): 504-512.
28. Boue SM, Wiese TE, Nehls S, Burow ME, Elliott S, Carter-Wientjes CH, Shih BY, McLachlan JA, Cleveland TE. Evaluation of the estrogenic effects of legume extracts containing phytoestrogens. Journal of Agricultural and Food Chemistry, 2003; 51(8): 2193–2199.

29. V De Leo, Lanzetta D, R Cazzavacca, G Morgante. Treatment of neurovegetative menopausal symptoms with a phytotherapeutic agent. Minerva Ginecologica, 1998; 5(5): 207-211.
30. Malinow MR, Bardana EJ, Pirofsky BJ, Craig S and McLaughlin P. Systemic lupus Erythematosus -like syndrome in monkeys fed alfalfa sprouts: role of a nonprotein amino acid. Science, 1982; 4-23; 216(4544): 415-417.