# Red Clover (*Trifolium pratense*) Benefits in Dentistry: A Narrative Review

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***Abstract* —** The herbal plant red clover (*Trifolium pratense*) includes four phenolic compounds, categorised as phytoestrogens which are a set of isoflavones comprising daidzein, biochanin A, genistein, and formononetin. These compounds are plant-derived and practically equivalent to 17-estradiol. This study reviewed the scientific literature reporting the benefits of red clover as medicament and herbs in the field of dentistry. The PRISMA checklist was used to conduct the systematic review in Web of Science and PubMed which retrieved 197 records. The records related to the benefit of red clover in dentistry were screened, and after removal of duplicates, 46 records were identified, reviewed and incorporated in this study. The *Trifolium pratense* extracts provide several dental advantages, including increased calcium levels in bones and teeth and decreased bone porosity. It has a wide effect on the gingiva by improving blood flow and enhancing blood circulation. Besides, these compounds can regulate the level of glucose in the blood and lipid markers, assist in improving symptoms from menopause and its hormonal changes, while having acceptable effects as an anti-inflammatory and improving the skin texture. Further, it has tangible effect in increasing the secretion of salivary glands, broad effect in reducing caries and additional beneficial effects as an antioxidant and anticancer with significant effects on the development of most cells. The multiple benefits of *Trifolium pratense* promote essential and beneficial effects in preserving oral health providing significant effect in treating gingival diseases and dental problems.

**Keywords—Dentistry, Herbal, Isoflavones, Osteoporosis, *Trifolium Pratense***

## 1 INTRODUCTION

The use of herbs or herbal medicines has become an alternative to treatment with chemical medicines, and alternative medicine field has become a widespread option in recent years (1). The stems, seeds or flowers of plants or their oils are used in the prevention of many diseases that affect the body in general and the mouth and teeth in particular (2). The use of crops, their products, wild herbs, and extracts has been used throughout history in the treatment of many dental diseases especially tooth decay and the use of herbal oils as an analgesic for toothache (3). Currently, much research has been conducted in identifying suitable herbs for their application in the therapy of oral illnesses and dental problems, and among these, red clover as herb that is grown in many countries (4).

Red clover with scientific name *Trifolium pratense* is a perennial plant that lives on leaves, normally surviving more than two years which is indigenous to Europe (5). In Western Asia, and the Middle East, it can grow in height of up to 20 to 80 cm which is trifoliate (three leaflets) and the leaves are 15 to 30 mm long with broad width ranging from 2 to 15 mm (6,7). Red clover contains plant-derived oestrogen-like compounds, which are identical to 17-estradiol as it contains four phenolic compounds classified as phytoestrogens; it is a group of isoflavones, (8) which include daidzein, biochanin A, genistein and formononetin (8). Each of these compounds benefits human health and thus the health of the mouth and teeth. Daidzein and genistein are considered to protect against chronic illnesses such as malignancy, osteoporosis, and ischemic cardiovascular disease (9). Biochanin has anti-inflammatory, antioxidant, anticancer, and neuroprotective properties (10–12). Formononetin has medicinal benefits such as antimicrobial, cardioprotective, anti-hyperlipidaemia, anti-tumour, anti-diabetic, neuroprotective and antioxidant properties (13–16). Red clover additionally contains many other compounds with anti-inflammatory effects

lowering the potential of diabetes and reducing cardiovascular disease (17). It also contains many other organic and inorganic substances including protein compounds and amino acids with multiple medical benefits which are widely used in herbal medicines (5,18).

## 2 METHODOLOGY

A search utilising Web of Science and PubMed for scientific publications regarding the medicinal benefits and applications of red clover were conducted from March 2023 to December 2023, and a total of 197 records pertaining to the herb's therapeutic applications in dentistry-related fields were found during the search. The 197 records were articles published from 2004 to 2023.

### 2.1 Study design and research strategies

The systematic review was carried out using 197 records that were chosen before applying the selection criteria based on the medical use of red clover in the fields of therapeutic dentistry and the fact that red clover oil has important effects on living tissues in the mouth, including hard and soft tissues, and removing the unwanted effects of the diseases based on the PRISMA guidelines (19) (Fig. 1).

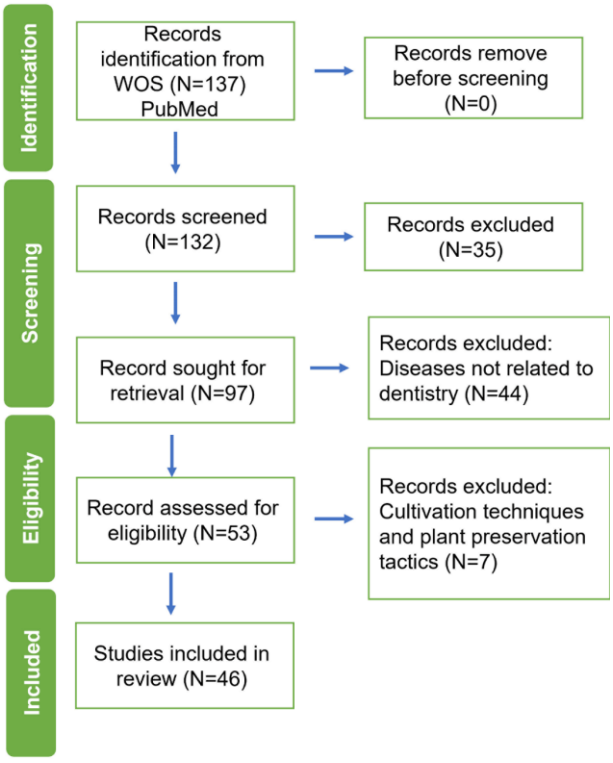


**Figure 1:** The PRISMA flowchart. Adapted from (19).

The research questions employed is this study was, (a) What are the benefits of red clover towards oral cavity?, (b) What are the benefits of red clover towards gingival and dental health? and, (c) How red clover demonstrates its advantages as a herbal therapy for dental condition? The keyword used in combination were and not limited to 'red clover', 'red clover and human', 'red clover oil and dentistry', 'red clover and tooth', 'red clover and gingiva', 'red clover and periodontal', and '*Trifolium pratense* and dentistry', '*Trifolium pratense* and gingiva', and '*Trifolium pratense* and tooth'.

### 2.2 Inclusion and exclusion criteria

The selection criteria were publications or research related to the effect of red clover extracts of leaves, seeds, or stems of the red clover plant on studies demonstrating the benefits of red clover oil components in the treatment or prevention of oral and dental pathologies, and benefits of red clover for somatic cells which are found in the oral cavity. Articles not published in English, and reporting only abstracts, repeat studies that did not support the objectives of this investigation, and non-availability of full texts were excluded. Additionally, research on cultivation techniques and plant preservation tactics was disregarded.

Therefore, the remaining articles included in this research were 46 articles which reports on animal's models, *in vitro* cell culture and clinical studies. The keywords from the selected articles were analysed using Visualisation of Similarities (VOS) software (Version 1.6.19, Leiden University, Netherlands) for analysing the commonly used keywords in red clover oil studies which were related to the current study.

## 3 RESULTS

After removing the excluded articles, the titles and abstracts were sorted. The full text of the remaining studies was read and concerned studies according to the qualification criteria were selected. Two authors (JGH & SNFMN) individually extracted the following information from the studies: author(s), year of publication, types of articles, selection of article methods, number of articles, evaluation properties, discussion, and results. A third author (AFM) eliminated any disagreements between the reviewers. Any disagreement relating to this process was resolved by the fourth author (IAK). Forty-six papers were ultimately selected for the

current review. The articles showed the effects of red clover extracts in both human and animal models. The red clover's effects on different oral tissues were highlighted, as well as how it can be used to prevent and treat a variety of diseases and pathological conditions, while promoting tissue immunity against the functional impairment brought on by the tissue changes because of the disease pathology. The current review also highlighted the various methods covered in these research articles including level-specific observations and experiments in human and animal, although not focus specifically on dentistry, but involving the effects that red clover may have on oral tissues.

Based on the keywords analysis using the VOS software (Version 1.6.19, Leiden University, Netherlands) on 97 records that were sought during retrieval, there were 2083 keywords, and after sorting, five clusters remaining with at least 50 items that form 585 links. It can be seen that 'gingivitis' and 'periodontitis' belong to two separate cluster that are interrelated with the keywords 'medicinal plant', 'herb', 'inflammation' and 'beneficial effect' that can be linked to 'women', 'oestrogen' and 'health'. The phytochemical constituents daidzein and formononetin were mostly tagged keywords based on the size of the circle as shown in Fig. 2 below.

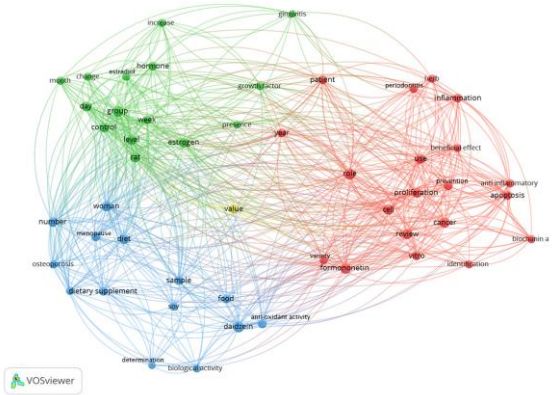


**Figure 2**: The network map based on VOS software (Version 1.6.19, Leiden University, Netherlands).

## 4 PHYTOCHEMICAL CONSTITUENT

According to the current scientific studies, red clover includes isoflavones which are plant components that produce oestrogen-like effects in the body (11), in addition to their anti-inflammation, antioxidant, and cancer prevention properties (10). Isoflavones from red clover have shown encouraging results in treating various symptoms of postmenopausal hormone imbalance, cardiovascular health, and osteoporosis with skin health and the proposed mechanism as reported previously (20). Isoflavones contain four phenol-like compounds: daidzein, biochanin A, genistein and formononetin (8,21).

### 4.1 Diazein

Dietary phytoestrogens like daidzein are hypothesised to safeguard versus ongoing illnesses general in most countries including osteoporosis, cancer and ischaemia affecting the cardiovascular system (14). It has a molecular formula of $C_{15}H_{10}O_4$ which belongs to the family of 7-hydroxyisoflavones which are 7-hydroxyisoflavones with an extra hydroxyl component located at location 4'. Daidzein is an anti-tumour drug; phytoestrogen, plant metabolite, EC 3.2.1.20 (alpha-glucosidase) blocker and EC 2.7.7.7 (DNA-directed DNA polymerization) antagonist (5,22).

### 4.2 Genistein

Menopausal symptoms, skin cancer, heart disease and melanoma have all been linked to genistein; according to epidemiological and experimental research (23), liver cancer model, genistein reduced serum alanine transaminase and aspartate transaminase levels (24). It has a molecular formula of $C_{15}H_{10}O_5$ which belongs to the 7-hydroxyisoflavone family, having extra locations 5 and 4' containing hydroxyl radicals; genistein is an antioxidant-rich flavonoid phytoestrogen. It functions as an anticancer drug, the tyrosine kinase blocker, EC 5.99.1.3 [DNA topping enzyme (ATP-hydrolysing)] blocker, oestrogen-like compounds, plant metabolite, neuroprotector, human molecule of urine which reflects the genistein acid combination (25).

Genistein is an isoflavone produced from the red clover plant. It is an antineoplastic and anticancer drug that blocks receptor tyrosine kinase with topoisomerase-II (type II DNA topoisomerases) action (26). It has been proven in experiments to elicit human beings as well as mouse lineages of cells that are arrested in the G2 phase (17). Furthermore, genistein is a kind of oestrogen-like compound that acts as a receptor for oestrogen modifier characteristics (27). It has been studied in clinical trials as an alternative to traditional

hormone treatment in postmenopausal women to help avoid cardiovascular disease (9).

### 4.3 Biochanin A

Biochanin A is a flavonoid originating from crops such as chickpeas, red clover and sorghum which is becoming increasingly popular due to its antioxidant, anti-inflammation, anticancer, and neurological qualities (11,28). It has a molecular formula ($C_{16}H_{12}O_5$) which is a 7-hydroxyisoflavone with an extra location 5' carboxylic compound with place 4' methoxides compound that belongs to the family of 7-hydroxyisoflavones (12). It is a phytoestrogen with potential advantages in cancer prevention (5). It belongs to the 7-hydroxyisoflavones and the 4-'-methoxy isoflavones and operates as oestrogen-like compounds, an agricultural metabolite, a substrate of EC 3.5.1.99 (fatty acid amide hydrolase), a tyrosine kinase blocker and a cancer-fighting substance (10).

### 4.4 Formononetin

Formononetin exhibits antibacterial, antioxidants, hyperlipidaemia protective properties, diabetic prevention, tumour prevention, cardio protective properties and neuroprotective among other things (29). It has a molecular formula of $C_{16}H_{12}O_4$ with 7-'hydroxyisoflavone containing a methoxide group located at site 4' which belongs to the 7-'hydroxyisoflavone family (15). It functions as a phytoestrogen as well as a plant metabolite and belongs to the 7-hydroxyisoflavones and the 4'-methoxyisoflavones and functions similarly to daidzein (16).

## 5 EFFECTS OF RED CLOVER ON HARD TISSUE

Several clinical and animal studies highlighted the positive effect of isoflavones in increasing bone mass and substantial anabolic impact as shown by alkaline phosphatase activity and bone levels of calcium (30). Many studies have found that genistein and daidzein have a significant effect on the process of bone remodelling and regeneration while increasing bone strength, and the anti-inflammatory effect of red clover can shorten the healing period in bone fractures (14). *Ex vivo* research on experimental rodents additionally proved that the daidzein substance increases the formation of collagen fibres (31). An experimental *in vivo* study on rats showed an increase and speed in the formation of collagen fibres in bone fractures of the rats that were treated with red clover oil compared to the control group which has not been treated with red clover oil. The red clover also has a positive effect on the teeth, by increasing the percentage of calcium in the blood's secretion while compensating for the lack of calcium ion in the hard tissues including the bones (30).

A study conducted in New Zealand in 1967 and published online in 2012 on sheep dental wear by feeding the sheep with Chou Moeller and red clover hay, and the wearing of anterior teeth was studied for eleven months in two poultry fields having a record of excessive wear. Supplemental forage was provided between eight and ten weeks during the winter, when grass growth was limited, and animals were starving. Their results showed that the peak of the teeth wear was reduced by more than 50% compared to the controls throughout this period. Over the course of the year, the wear of the incisor teeth decreased by approximately 57% (32).

In another study on ewes that was conducted in France showed that the application of red clover in food reduced erosion of the enamel layer that protects the teeth more than other ewes that were fed on a mixed diet of corn and chestnuts. According to the study, the enamel layer that protects the teeth in the ewes fed on red clover were more complex in terms of texture and stronger, even by a slight difference, but it remained significant (33).

According to research conducted on guinea pigs published in September 2016, the isoflavone genistein increases surface tension of the teeth through higher hardness value during the tooth movement process while undergoing orthodontic procedure. The study reported that the isoflavone genistein increases the odontoblast number, in addition to the obvious elevation in osteoblast and osteoclast cells which allows bone remodelling, and easy movement of the teeth during the orthodontic process. It is believed that the isoflavone genistein performs double action by strengthening the teeth and increasing the bone remodelling process (34).

Daily treatment of daidzein significantly reduced ligature-induced alveolar bone resorption, and enhanced bone volume production, while restoring the ligature induced degradations in trabecular bone micro-architecture metrics such as trabecular thickness, bone mineral density, trabeculae separation and structure model index

(23). The study also demonstrated the significant effect of daidzein on fibroblast cells by increasing their cell division and differentiation resulting in significant effect on bone formation (23).

## 6 INFLUENCES OF RED CLOVER ON GINGIVAL TISSUE AND PERIODONTIUM

Much research has indicated that the herb red clover is beneficial and has an effective therapeutic effect such as anti-inflammatory property and effect on improving blood circulation (8). These two elements have a clear effect on the health of gingiva and periodontium (20). Inflammation of the gingiva can give rise to gingivitis, also known as an infection of the gums where the gingival tissue swells and bleeds which if left untreated for a long time, leads to development of gingival ulceration and possibly tooth loss (35).

The anti-inflammatory effect of red clover assists in reducing the destructive effect of gingivitis, reduce gingival bleeding and congestion, reduces the breakdown of periodontal ligaments, moreover, the phytoestrogen presents in red clover, which is an isoflavone, has a significant effect on gingival tissue (6). The physiological effect of phytoestrogens on the gingiva can be observed when oestrogen concentrations in the blood is less than 100 pg/ml. Clinical administration of aromatase to the animal during the study period leads to development of inflammatory gingival swelling and increase in size. Nevertheless, when oestradiol is administered back, the gingival condition improved clinically, and their findings suggested that phytoestrogens have a significant impact on physiological events in the gingiva such as direct or indirect cellular proliferation and differentiation (36).

Another study found that the amount of oestrogen in the body influences gingivitis and has a direct effect on bacterial plaque especially among pregnant women. High level of oestrogen among pregnant women leads to high bleeding on probing and visible plaque index especially during second and third trimesters (37). Among the classic studies that were conducted during the earliest eighties of the 20$^{th}$ century was the Jensen's research (38), in which the direct relationship between oestrogen and bacterial plaque was proven, as any imbalance in the secretion of the hormone, whether by an increase or a decrease, could lead to an increase in the bacterial number, thus, employing red clover or red clover oil as a supplement as an important nutritional supplement for postmenopausal women. Besides, due to red clover benefits such as collagenase enzyme inhibitory activity that leads to enhancement of gingival collagen synthesis especially from the red clover flowers, this study suggested usage of red clover as an herbal product for mouthwash especially among menopausal women (20).

A proven study published in 2018 highlighted the effect of flavonoids on gingival tissues and cells through cellular biology and rodent experiments in real life investigations, including modulation of reactions to inflammation in gingival parts and putative preserving outcomes for periodontal ligaments and alveolar jaw structures. Flavonoids anti-inflammatory activity may inhibit progression of periodontitis through pathogen-associated molecular pattern (PAMP) proteins that binds to Toll-like receptor (TLR) by targeting the TLR signalling pathway. Flavonoids have been demonstrated to have potentially favourable effects on a variety of periodontium tissue cells, comprising epithelial periodontal cells, gingival fibroblasts, periodontal ligament fibroblasts and alveolar bone repair osteoblasts and according to the data, flavonoids are incredibly promising medicinal products for both prevention and therapy of periodontitis that could be easily supplied (31). As previously shown in the study of Bae, the significant effect of daidzein on increasing division and distinguishing of fibroblast cells, this effect extends to the periodontium since fibroblast cells are the primary cells that handle the formation and repair of connective tissue ligaments distributed around the teeth (23). This was also shown by a previous study published in 2006 that carried the same results on the significant effect of daidzein on fibroblast cells (24).

As for Bhattarai, a rat's experiment published in 2017, the outcome of genistein on alveolar bone and on periodontium was demonstrated through X-ray microtomography and histological investigations demonstrated that an intraperitoneal injection of genistein (20 mg/kg body weight) daily for a period of three weeks prevented lipopolysaccharides (LPS) mediated mandibular bone loss and periodontal tissue damage degeneration. In addition, genistein plummeted osteoclast production and the expression of inflammation-related biomarkers in inflamed tissue area of periodontitis animals; 30-

70 µM genistein therapy effectively inhibited osteoclast development and reduced the number of osteoclast-specific proteins in receptor activator of nuclear factor B ligand (RANKL)-stimulated osteoclast development in macrophages or LPS-stimulated macrophages. Genistein inhibited the expression of inflammation-related markers in both LPS-stimulated macrophages and human gingival fibroblasts in a dose-dependent manner furthermore, genistein at 50 µM protected human gingival fibroblasts from LPS-mediated stressors as in mitochondria dysfunction as well as cellular reactive oxygen species buildup, however, its protection was severely reduced (25).

## 7 EFFECTS OF RED CLOVER ON SALIVARY GLANDS

The glands that secrete saliva execute a crucial function essential and significant impact on wellness of the mouth and teeth through self-cleaning of the teeth and a buffer effect by reducing acids that lead to tooth decay, in addition to its important role as an antimicrobial and an inhibitor of plaque aggregation (39). Therefore, any defect in the production of saliva in terms of quantity or quality can lead to catastrophic problems for the mouth and teeth. Thus, researchers have been concerned in many research papers with the significant effects of herbal materials on preservation of salivary glands and among those herbal substances with a positive effect of isoflavones (40).

The consequences of isoflavones on impaired salivary production were investigated among individuals with dry mouth for two months where isoflavone aglycones were supplied at a daily intake of 25 mg to 15 volunteers with an average age of 67.9 ± 8.0 years, and salivary secretion was measured. Based on the salivary flow rate and a self-completed questionnaire, the results revealed a considerable improvement, suggesting that isoflavones can alleviate with the symptoms of salivary gland hypofunction (41).

The significant effect of isoflavones on the submandibular gland were analysed on 96 ovariectomised rats and compared with 24 non-ovariectomised rats that were given one of four treatments: 17-estradiol, isoflavone extract, a combination of both or water as a placebo. Histomorphometry analysis was performed on the submandibular glands and the results demonstrated that hormone insufficiency influenced and reduced the number of acini and ducts of ovariectomised rats in comparison to the sham group. In comparison to the group receiving placebos, all therapies increased the number of ducts and acini. Since ovariectomy lead to a reduction in the number of glandular acini and ducts, it was proposed that oestrogen deficiency may be the cause connected to salivary gland function, thus, treatment with oestrogen, isoflavones, or a mixture of the two proves successful in lowering the effects of ovariectomy on the salivary glands (26).

The antioxidant and anticancer role of isoflavones genistein was assessed on human salivary gland (HSG) cell lines where it was reported that combining isoflavones with gamma rays allow the cancer cells to become more sensitive by arresting the cell cycle at G2/M phase, and cells count that received genistein dropped in a concentration-dependent manner as compared to the untreated group (40).

A study showed that Biochanin A, which is one of the components of isoflavones, has a significant effect on the formation and evolution of the submandibular gland from female rat offspring that produces saliva and upregulated the oestrogen receptor β while acting as growth hormones (42). This finding may highlight the beneficial effects on Biochanin A in increasing salivary production among women with dry mouth.

## 8 EFFECTS OF RED CLOVER ON ORAL TUMOUR

In an effort to confirm red clover's biological function, in terms of its anti-carcinogenesis especially the carcinogens of hormonal imbalances, many studies showed that the isoflavones present in red clover is commonly used as a drug to relieve the symptoms of menopause for women (16). These isoflavones were able to deal with the receptor of aryl hydrocarbons (AhR), in terms of its ability to activate it (5). Since Aryl hydrocarbon receptors (AhR) activation disturbs the cell cycle and promotes cell death, selective Aryl hydrocarbon receptors modulators were previously related to cancer therapy and mitigation, particularly in hormone-dependent tumours, which mostly involved *in vitro* studies with many different types of cells (5). The triggered Aryl hydrocarbon receptors collaborates with the oestrogen receptor, and it is shown that formononetin and

biochanin A is a potent agonist for AhR where these isoflavones are 10 times stronger compared to the indole compounds, indole-3-carbinol and diindolylmethane, which have been demonstrated to be significant AhR antagonists, with $EC_{50}$ levels of $5.8 \times 10^{-6}$ and $1.1 \times 10^{-6}$ mol/l, respectively (29).

In a study by Mu, on ovariectomised mice, they showed that formononetin has substantial ability to neutralise free radicals and formononetin injection can inhibit the progression of lipid peroxidation by boosting both enzymatic and non-enzymatic defences against antioxidants. The inhibiting effect of formononetin on lipid peroxidation might be attributed at least in portion, to its activity on enzymes that are antioxidants and the non-enzymatic system, which eliminates free radicals and so has an anti-tumour influence (43).

The oxidised metabolite of genistein and daidzein demonstrated the highest antioxidant capacity; despite a few limitations, they were more efficient than the positive controls containing quercetin and ascorbic acid. Formononetin, the 4'-O-methyl ether of daidzein, has little antioxidant activity, considering the antioxidant efficacy of isoflavones in the role of antioxidants is observable at concentrations far within the range observed in the plasma of individuals eating red clover nutrient supplements, this biologic function may be of physiologic relevance (44).

The potential of red clover isoflavones to decrease COX enzymes towards RAW 264.7 mouse macrophage cell line and human monocytes were assessed, and both demonstrated that isoflavone at a concentration of 1-100 µM significantly suppressed the synthesis of prostaglandin E2 and/or thromboxane B2 in RAW 264.7 cell lines and in human monocytes. Thus, it is possible that the lower prevalence of different malignancies in areas with high dietary isoflavones consumption is attributable to COX restriction (45).

## 9 EFFECTS OF RED CLOVER ON CELLS IN VITRO

The red clover extracts promote endothelial-derived nitric oxide (NO) generation by cells of the endothelial system activating transcriptional pathways but are incapable of stimulating fast endothelial-derived nitric oxide (NO) synthesis via no genomic processes. Red clover extracts increase the expression and the function of endothelial nitric oxide (NO) synthase across long-term exposure so that, the effects are mediated by the recruitment of oestrogen receptors, furthermore, isoflavones produced from red clover work synergistically with 17-estradiol in enhancing nitric oxide production in endothelial cells synthesis (46).

In a study, rats with ovariectomy showed decreased bone mineral material, femur weight, femur density, the strength of the tibia, and raised amount of bone specific alkaline phosphatase and the number of osteoclasts in femoral segments. Interestingly, treatment with isoflavones improved the minerals in bones material, tibia rigidity, femur volume, femur density, and conserved blood alkaline phosphatase levels from rising. Furthermore, as compared to the ovariectomised control rats, isoflavones administration dramatically reduced the quantity of osteoclasts. These data imply that red clover isoflavones are beneficial in preventing ovariectomy related decreased bone density, most likely by decreasing bone turnover via bone resorption resistance (47).

Red clover isoflavones can boost liver efficiency by stimulating hepatic Kuepfer cells (special macrophages found in the liver), alongside to their obvious influence on monocyte cell function (6). It was shown that 14-week therapy with a red clover extract standardised to contain 11% isoflavones as assessed by HPLC improved the morphology of the epidermis, epidermal thickness, and the quantity of total collagen evaluated by colorimetric analysis in ovariectomised rats. The skin of ovariectomised rats administered red clover isoflavones (20 and 40 mg total isoflavones daily for 14 weeks) appeared to be well-organised, with an ordinary epithelium of a consistent thickness and typical keratinisation; circulation, collagen, and elastic filaments were dramatically generated, and the percentage of fibroblast cells enhanced as compared to the control group that received no treatment (48).

In another study by Huang and co-worker (49), isoflavones Portion 3 which comprises aglycones (daidzein, genistein) plus acetyl glucosides (acetyl-daidzein, acetyl-genistein isoflavones) may be able to suppress UV-induced keratinocyte death of cells, reducing skin elimination, trans epidermal loss of water, and erythema along with epidermal thickening. Furthermore, topical application of Portion 3 increased catalase activity in mice exposed to ultraviolet (UV) simultaneously inhibiting the synthesis of cyclooxygenase-2 (COX-2) and proliferating cell nuclear antigen (PCNA), in comparison with ISO-1 and genistein.

Animal receiving Portion 3 demonstrated much better protective effects versus UV-induced oxidative damages and keratinocyte mortality than the remaining fractions, making isoflavones extract Portion 3 a viable anti-photo aging agent for dermatological use (49).

## 10 CONCLUSIONS

Alkaline phosphatase activity and calcium levels in the bone are two indicators of the significant anabolic impact and beneficial effect of isoflavones on growing bone mass that have been demonstrated *in vivo* or *in vitro* by several clinical and animal investigations (30). As an efficient anti-inflammatory and blood circulation enhancer, red clover has a significant positive impact on living cells and tissues (8). Furthermore, gingivitis is an infection of the gingiva that causes swelling of the gingival tissue. Daidzein has a significant effect on fibroblast cell division and differentiation, which affects the periodontium because fibroblast cells are the main cells responsible for the formation and maintenance of the connective tissue ligaments that surround teeth (23).

Studies have also demonstrated the beneficial effects of isoflavones on salivary glands and increased salivation, which is important since saliva protects the gingiva and moisturises the mouth while also helping to prevent caries through the buffer system (26). Another study has shown that red clover has an antitumor and anticancer effects (44). Formononetin has a strong anti-free radical effect and that injecting it into rats assist in retarding the lipid peroxidation from developing by strengthening their enzymatic and non-enzymatic defences against antioxidants. As a result, lipid peroxidation is at least responsible for the inhibitory impact of formononetin, due in part to its action on the non-enzymatic system and antioxidant enzymes, which scavenge free radicals and have an anticancer impact (43). The isoflavones from red clover as indicated from Portion 3, which is composed of acetyl glucosides (acetyl-daidzein, acetyl-genistein isoflavones) and aglycones (daidzein, genistein), may be able to inhibit DNA damage caused by UV light (49).

Red clover has many benefits towards oral and dental structures as shown by the mentioned studies and further exploration of the red clover in *in vivo* animal studies especially related to the use for enhancing bone regeneration following tooth loss or bone trauma is very much anticipated.

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