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**Review Article**


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## PHARMACOLOGICAL ACTIVITY OF SPINACIA OLERACEA LINN.- A COMPLETE OVERVIEW

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### ABSTRACT

Herbal and natural products of traditional medicine have been used for centuries in every culture throughout the world. Medical professionals and Scientists have shown increased interest in this field as they diagnose the true health benefits of these remedies. "Let food be your medicine and let medicine be your food" was advised by the father of medicine, Hippocrates, over two million ago. It's still true today that "you are what you eat." Spinach is a leafy green vegetable that came originally from south-western Asia and is now grown in most parts of the world. Scientifically it is known as *Spinaciaoleracea*Linn. (Family-*Chenopodiaceae*). Though Spinach is most often used as a food, it has medicinal value as well. Spinach is packed with vitamins such as vitamin C, vitamin A and vitamin E and minerals like magnesium, manganese, iron, calcium and folic acid. Spinach is also a good source of chlorophyll, which is known to aid in digestion. Spinach is also rich in the carotenoids beta-carotene and lutein. It is a good source of the bioflavonoid quercetin with many other flavonoids which exhibits anti-oxidant, ant proliferative, anti-inflammatory, antihistaminic, CNS depressant, protection against gamma radiation, hepatoprotective properties in addition to its many other benefits. Spinach is also used to prevent the bone loss associated with osteoporosis and for its anti-inflammatory properties in easing the pain of arthritis. Spinach is good for the heart and circulatory system and has energy-boosting properties. Spinach is truly one of nature's most perfect foods.

**Key words:** *Spinaciaoleracea*, Spinach, Vegetable, Antioxidant, Flavonoids.

### INTRODUCTION

A high intake of fruit and vegetables is well known to have positive effects on human health and has been correlated to a decreased risk of most chronic diseases such as cardiovascular disease, diabetes and several forms of cancer<sup>[1, 2]</sup>. *Spinaciaoleracea* is commonly known as Spinach (English), Chhurika (Sanskrit), Palak (Hindi; Gujarati; and Marathi), Palakh (Kashmiri), Palang (Bangla), Pasalai (Tamil), and Mathubucchali (Telugu) The<sup>[3]</sup>.

In different traditional medicinal system it is known by different names. Its Ayurvedic name is 'Paalankikaa', in 'Unani' it is called as 'Paalak', where as in 'Siddha' it is known by 'Vasaiyila-keerai'<sup>[4]</sup> Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, iron. *Spinaciaoleracea* is an edible flowering plant in the family of *Amaranthaceae*. It is an annual plant (rarely biennial), which grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions<sup>[5]</sup>. Apart from having nutritional value, it has been also credited with various biological activities like virus inhibitor<sup>[6]</sup>, anthelmintic<sup>[7]</sup>, antioxidant

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[8], hepatoprotective<sup>[9]</sup> and reducing risk of breast cancer<sup>[10]</sup>.

General information of *Spinacia Oleracea*  
Linn

### Scientific Classification

Plantae

Subkingdom : Tracheobionta

Superdivision: Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Caryophyllidae

Order : Caryophyllales

Family : Chenopodiaceae

Genus : *Spinacia* L.

Species : *Spinaciaoleracea*L.<sup>[11]</sup>

### BOTANICAL DESCRIPTIONS

**Stem:** Erect from 30-60 cm high, round, smooth, piped, succulent, sometime reddish.

**Leaves:** Alternative, the lower ones very long petioled, variously lobed with lobes of an acute triangular shape, smooth on both the side.

**Flowers Male-** Flowers on long terminal glomerate spikes and on shorter ones from the axial, very numerous, sessile, calyx 4-parted, stamen 4, anthers twin, very large.

**Female-** Flowers axillary, sessile, crowded. Calyx 2-tipped with a projecting horn in each side, growing into spines when the seed is ripe. Styles generally 4, white tapering. Capsule 1-celled, 1-valved, armed, with 2 opposite short horns, and crowned with the small remaining calyx<sup>[3]</sup>  
(Figure 1)



Figure 1: *Spinaciaoleracea*

### CHEMICAL CONSTITUENTS

#### Flavonoids:

*Spinaciaoleracea* is very rich in the flavonoids. Various flavonoids reported to be present are quercetin; myricetin; kampeferol<sup>[12]</sup>; apigenin; luteolin; patuletin; spinacetin; jaceidin; 4'-glucuronide; 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide; 5,4'-dihydroxy-3,3'-dimethoxy-6:7-methylene dioxyflavone-4'-glucuronide<sup>[13]</sup>; 5,4'-dihydroxy-3,3'-dimethoxy-6,7-methylene-dioxyflavone

(C<sub>18</sub>H<sub>14</sub>O<sub>8</sub>.); 3,5,7,3',4'-pentahydroxy-6-methoxyflavone<sup>[14]</sup>.

#### Phenolic Compounds:

The polyphenols isolated from the plant are *para*-coumaric acid, ferulic acid, *ortho*- coumaric acid<sup>[15]</sup>.

#### Carotenoids:

Spinach shows presence of different carotenoids like lutein,  $\beta$ -carotene, violaxanthin and 9'-(Z)-neoxanthin.

#### Vitamins:

*Spinaciaoleracea* contains high concentration of vitamin A, E, C, and K. and also folic acid, oxalic acid.

**Minerals:** Along with these chemicals various minerals present in the spinach. These are magnesium, manganese, calcium, phosphorus, iron, zinc, copper and potash<sup>[14]</sup>.

## DISTRIBUTION

Native to South- west Asia; cultivated throughout India<sup>[4]</sup>.

## BIOLOGICAL REVIEW

*Spinaciaoleracea* L.(Chenopodiaceae), commonly known as “Spinach” in English and “Palak” in Hindi, found throughout India, and used in inflammation of liver & in jaundice.

The leaves are used for bowel and lung inflammation, febrile affliction and cooling.

Spinach improves cerebellar physiology and motor learning in aged rats.

## CHEMICAL REVIEW

Spinach is a rich source of carotenoid (lutein,  $\beta$ -carotene, and zeaxanthin), p-Coumaric acid, ascorbic acid, proteins, vitamins and other substances. The

spinach is well reported to be a good source of minerals, vitamin B-complex, ascorbic acid, flavonoids, and apocyanin.

Spinach, as a potherb, is rich in nitrogenous substances, hydrocarbons and iron sesqui-oxide.

The chemical composition of *Spinaciaoleracea* is calcium-73mg/100gm, magnesium-84mg/10gm, iron-10.9%, phosphorus-1mg/100gm, potassium-206mg/100gm and vitamins C,A,thiamine,riboflavin,lutein and zeaxanthin.

Aerial parts afforded rutin, hyperoside, astragalin and caffeic, chlorogenic, neochlorogenic and protocatechuic acids. Seeds contain glycoprotein- bound hexosamine. Roots contain spirasaponins.

It also contains patuletin, quercetin, saponin, catechol and sterols. The sterols reported in seeds of this plant are Stigmasterol, Spinatoside, 20-hydroxyecdysone etc.

Spinach is regarded as a valuable dietary source of vitamin A, nonheme iron, folate, and lutein. Spinach also contains oxalates and nitrates that may have potential negative effects.

## Different Activity Reported for Spinach

Sr No.	Reported Activity	Author(s)	Publication
1	Scavenging of reactive oxygen species by a novel glucurinated flavonoid antioxidant isolated and purified from spinach	Margalit Bergman Alexander Perelman, et al	Phytochemistry 62 (2003) 753-762. <sup>[16]</sup>
2	Antibacterial Activity of Aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus Species.	Jignaparekh, Sumitra V, et al	Turk J Biol 32 (2008) 63-71. <sup>[17]</sup>
3	<i>SpinaciaOleracea</i> modulates radiation induced biochemical changes in mice testis.	RashmiSisodia*, RituYadav,et al	Indian Journal of Pharmaceutical Sciences, June 2008. <sup>[18]</sup>
4	Effect of Hexane extracts of Spinach in the removal of Arsenic from rat.	Badar Uddin Umar	Bangladesh J Pharmacology 2007; 2: 27-34. <sup>[19]</sup>

5	Amelioration of CCl <sub>4</sub> – induced Hepatosuppression by Spinaciaoleracea L. Leaves in wistar Albino rats	R.S. Gupta* et. al	Pharmacology online 3:267-278 (2006). <sup>[20]</sup>
6	Dietary Supplementation with Blueberries, Spinach or Spirulina reduces Ischemia brain damage.	Yu Wanga, Chenfu, et al	Experimental Neurology 193 (2005) 75-84. <sup>[21]</sup> <a href="http://www.Elsevier.com">www.Elsevier.com</a>
7	Role of Spinaciaoleracea as Antioxidant: A Biochemical Study on Mice brain after exposure of Gamma Radiation	Rajesh Kumar, et al	Asian J.Exp. Sci, Vol.17, No.1&2, 2003, 51-57. <sup>[22]</sup>
8	A natural Antioxidant Mixture from spinach does not have Estrogenic or Antiestrogenic Activity in Immature CD-1 mice	Elizabeth Padilla bank* , Wendy N. Jefferson*, et al	Nutrient Interactions & Toxicity research communication, the Journal of nutrition,2003. <sup>[23]</sup>
9	Eighteen month old fischer 344Rats fed a Spinach Enriched Diet show improved delay classical eye blink conditioning and reduced expression of TNF and TNF in the cerebellum.	M.ClaireCartford et al	The journal of Neuroscience , July 15,2002,22(14):5813-5816. <sup>[24]</sup>
10	Iron application Ameliorates Copper Toxicity Effects In Spinach	Nautiyal, N.; Chatterjee,C,et al	Indian journal of Plant Physiology (India) 0019-5502 V.7(2) P. 198-200,2002. <sup>[25]</sup>
11	Spinaciaoleracea L. protects against gamma radiations: A study on glutathione and lipid peroxidation in mouse liver.	A.L. Bhatia*, M. Jain, et al	Phytomedicine 11 (2004) 607-615. <sup>[26]</sup> <a href="http://www.elsevier.de/phymed">www.elsevier.de/phymed</a>
12	Bioactive compounds in Baby Spinach (Spinaciaoleracea L.) effects of pre and postharvest factors.	Sara Bergquist	Doctoral thesis Swedish University of Agricultural Sciences Alnarp 2006. <sup>[27]</sup>
13	Effect of Boiling and storage on Beta-carotene content of Different vegetables.	Farida Anjum, et al	Pak. J. life soc. Sci. (2008)6(1):63-67. <sup>[28]</sup>
14	Topical & oral administration of the natural water soluble antioxidant from spinach reduces the multiplicity of Papillomas in the Tg.AC mouse model	A.Nyska, et al	Toxicology Letters 122 (2001) 33-44 <sup>[29]</sup> <a href="http://www.elsevier.com/locate/toxlet">www.elsevier.com/locate/toxlet</a>
15	Determination of Vitamin C,β-carotene and Riboflavin contents in five	Amin Ismail &CheahSook Fun	Mal J Nutr 9(1):31-39, 2003. <sup>[30]</sup>

	green vegetables organically and conventionally Grown		
16	In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae	Parekh j and Chanda S*	African Journal of Microbiology Research Vol. 1 (6) pp. 092-099 November-2007. <sup>[31]</sup>
17	Spinach for sight : Light sensitive proteins in chlorophyll may provide treatment for some eye diseases	Eli Greenbaum, et al	Oak Ridge National Laboratory , September 2001. <sup>[32]</sup>
18	CNS depressive role of aqueous extract of Spinaciaoleracea leaves in adult male albino rat	Sutapa Das &DepajaniGuha	Indian Journal of experimental biology, Vol 46,2008, 185-190. <sup>[33]</sup>
19	Possible Antitumour Promoters in Spinacia Oleracea and comparison of their content among cultivators	Rong Wang , Toshio Furumoto.,et al	Biosci. Biotechnol. Biochem, 66 (2), 248-254,2002. <sup>[33]</sup>
20	Immunization against rabies with plant derived antigen	Anna Modelska, VidadiYusibov*, et al	Proc. Natl. Acad. Sci. USA Vol.95, pp. 2481-2485, March 1998 Immunology. <sup>[34]</sup>
21	Effects of Antioxidants Apocynin and the Natural water soluble antioxidant from spinach on cellular damage induced by Lipopolysaccaride in the rat	Liat L1* et al	Toxicologic Pathology, Vol 28 No.4. pp . 590-587, 2000. <sup>[35]</sup>
22	Ecdysone 20 monoosygenase , a cytochrome P450 enzyme from Spinach, Spinaciaoleracea Linn	Robert J. Grebenok*, et al	Photochemistry, Vol.42 No.4,pp 927-933, 1996 1996 Elsevier Science Ltd. <sup>[36]</sup>
23	Antioxidant Mixture from Spinach does not have Estrogenic or Ant estrogenic activity in Immune CD-1 mice	Lomnitski, L., Padilla-Banks, et al	American Society for Nutritional Sciences.2003. <sup>[37]</sup>

### Mechanism of Action by constituents of S. Oleracea

- Hepatoprotective activity of S.oleracea Linn. Is based on its active constituents, including  $\beta$ -carotene, lutein, zeaxanthine, flavonoids, vitamin C, p-coumaric acid and micronutrients.
- Free radical scavenging compounds such as  $\beta$ -carotene and vitamin C can protect DNA from oxidizing radical reactions.
- $\beta$ -carotene is a potent free radical quencher, singlet oxygen scavenger, and lipid antioxidant.
- $\beta$ -carotene has already been reported to quench not only singlet oxygen but also

to scavenge a variety of free radical species.

- Zeaxanthine is only as effective as  $\beta$ -carotene in inhibiting autooxidation of lipids in solution, but is about 50% more effective in retarding hydroxide formation in phosphatidylcholine liposomes.
- Lutein is effective at inhibiting autooxidation of cellular lipids.
- Vitamin C is considered to be the most important antioxidant in extracellular fluids.
- It acts to protect membranes against peroxidation by enhancing the activity of  $\alpha$ -tocopherol, the chief lipid soluble and chain breaking antioxidant.
- Flavonoids are typical phenolic compounds and reported that LPO can be inhibited by flavonoids, possibly through their activity as strong  $O_2$  – scavengers (Baumann J, et al,1980) and singlet oxygen quenchers.
- P-Coumaric acid derivatives are strong antioxidants and have a ability of scavenge free radicals.

### TRADITIONAL USES

The plant is sweet, cooling, carminative, laxative, alexipharmic; useful in diseases of blood and brain, asthma, leprosy, biliousness; causes “kapha” (Ayurveda). It has been used in the treatment of urinary calculi. In experiments it has been shown to have hypoglycaemic properties. The leaves are cooling, emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestible, anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soalding urine, arrest vomiting, biliousness, flatulence. And have been used in the treatment of febrile conditions. The seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, and diseases of the brain and of the heart (Yunani). Seeds are laxative and cooling. They have been used in the treatment of difficulty in breathing, inflammation of the

liver and jaundice. The green plant is given for the urinary calculi<sup>[3,38]</sup>.

## PHARMACOLOGICAL ACTIVITIES

### Antioxidant Activity

The chemical fraction of natural antioxidant (NAO) components in *Spinaciaoleracea* was reported by Grossman in 2001. Spinach leaves were extracted with water and the 20,000 g supernatant which contained the antioxidant activity was extracted with water: acetone (1:9) solution. The 20,000 g supernatant obtained was further purified on reverse phase HPLC using C-8 semi-preparative column. Elution with 0.1% TFA resulted intensive hydrophilic peaks. Elution with acetonitrile in TFA resulted in seven additional hydrophobic peaks. All the peaks were detected at 250 nm. All the fractions obtained showed antioxidant activity when tested using three different assays. Based on  $^1H$  and  $^{13}C$  NMR spectroscopy four of the hydrophobic fractions were identified as glucuronic acid derivatives of flavonoids and three additional fractions as Trans and cis isomers of p-coumaric acid and others as meso-tartarate derivatives of p-coumaric acid. The study demonstrated for the first time the presence of both flavonoids and p-coumaric acid derivatives as antioxidant components of the aqueous extract of spinach leaves<sup>[39]</sup>.

### Protection against Gamma Radiation

The protective effect of 1100 mg/kg/day of 50% methanolic extract of *Spinaciaoleracea* L. (MESO) against radiation-induced oxidative stress were evaluated in terms of lipid peroxidation (LPO) product and tissue levels of glutathione. The animals were exposed to gamma radiation at a rate of 1.07 Gy/min with a source-to-surface distance of 77.5 cm. The animals were autopsied at 1, 3, 7, 15 and 30 days post-exposure. LPO increased after irradiation up to day 15 in the untreated-irradiated mice and up to day 7 in MESO pre-treated irradiated mice. LPO values were significantly lower in the MESO pre-treated irradiated mice as

compared to respective untreated-irradiated mice at all intervals, which reached normal values from day 7 onward. It was found that radiation-induced augmentation in malondialdehyde contents and depletion in glutathione changes in liver can be altered by MESO. The protection may be attributed to the combined effects of its constituents rather than to any single factor as the leaves are rich in carotenoid content (β-carotene, lutein, Zeaxanthine), ascorbic acid, flavonoids and p-coumaric acid<sup>11</sup>. The radioprotective efficacy of spinach against radiation induced oxidative stress was studied by Verma and Bhatia in 2003. For the experiments, Swiss albino male mice treated with *Spinaciaoleracea* leaves alcoholic extract (SE) once daily at the dose of 1100 mg/kg/day p.o. for 15 days. The animals are exposed to single dose of 5 Gy of gamma radiation at the dose rate of 1.07 Gy/min. After the exposure mice were sacrificed at different autopsy intervals viz. 1, 3, 7, 15 and 30 days. Brain was removed and processed to estimate LPO. Radiation induced significant elevation in the LPO values, which were lowered by supplementation of SE prior to irradiation at all the intervals studied. The protection rendered with SE in LPO value of brain in this study indicates the possible role *Spinaciaoleracea* as radio protector to some extent if taken continuously which might be due to synergistic effect of antioxidant constituents present in the spinach<sup>[40]</sup>.

### Anticancer Activity

In one study spinach ethanol extract (SE) and the three fractions by the hydrophobic column chromatography were investigated for their inhibition of calf, DNA polymerases (pol). The spinach glycolipid fraction dose dependent inhibited the activity of pol α with IC50 value of 43.0 μg/ml and the fat soluble fraction slightly inhibited the activity of pol α, although the water soluble fraction did not show such an effect. The ethanol extract from spinach had no effect on pol α, although the extract contains pol inhibitory glycolipid. This concluded that the spinach

glycolipid fraction can inhibit mammalian pol activity, human cultured cancer cell growth, and in vivo solid tumor proliferation with oral administration. This fraction could help to prevent cancer and be a functional food with anticancer activity<sup>[41]</sup>.

### Inhibition of Mammalian DNA Polymerases

The purification of the major glycolipids in the class of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG), from green vegetable spinach (*Spinaciaoleracea* L.) was reported. MGDG was an inhibitor of the growth of NUGC-3 human gastric cancer cell, but the DGDG and SQDG has no cytotoxic effect. So researcher studied MGDG and its monoacylglycerol-form, monogalactosylmonoacylglycerol (MGMG) in detail. MGMG with one fatty acid molecule was obtained from MGDG with two fatty acid molecule by hydrolyzing with the pancreatic lipase. MGMG was also found to prevent the cancer cell growth. MGDG was the potent inhibitor of replicative DNA polymerases such as α, δ and ε. MGMG inhibited the activities of the all mammalian DNA polymerases including repair-related DNA polymerases β with IC50 value of 8.5-36 μg/ml and the inhibition by the MGMG was stronger than that by the MGDG. Both the MGDG and MGMG could halt the cell cycle at G 1 phase, and subsequently induced severe apoptosis<sup>[42]</sup>.

### Sulphite Oxidase Activity

The spinach chloroplasts possess a sulphite oxidase activity coupled with oxygen consumption and reduction of ferricyanide. This activity is associated with thylakoids and solubilized by non-ionic biological detergents. The pH and temperature dependencies of sulphite oxidase activity solubilized by Triton X-100 from spinach thylakoids were consistent with those of an intrinsic membrane protein. This isolated activity was insensitive towards radical scavengers (mannitol, mannose and



fructose) and catalase, and was inhibited only with very high concentrations of superoxide dismutase. Thus, observed sulphite oxidation was not induced through the photosynthetic electron transport system, but achieved via a thylakoid membrane enzymic system showing a sulphite oxidase activity. Kinetic parameters of thylakoid sulphite oxidase were measured and compared with those of other sulphite oxidases<sup>[43]</sup>.

### Hepatoprotective Activity

Gupta and Singh 2006 reported the amelioration by *Spinaciaoleracea* L. leaves alcoholic extract (SE) against the hepatosuppression induced by carbon tetrachloride (CCl<sub>4</sub>). This was evaluated in terms of serum- marker enzymes like GGT, AST, ALT, LDH, SDH, GDH, ALP, serum-total bilirubin and total protein levels along with concomitant hepatic-antioxidants like SOD, CAT, GSH, GPx, GR, GST, ascorbic acid (vitamin-c),  $\beta$ -carotene and cytochrome P-450 enzyme. Whereas, LPO was monitored in both serum and liver. These biochemical parameters were significantly ( $P < 0.001$ ) altered by the single dose of CCl<sub>4</sub>. Pre-treatment with SE prior to the administration of CCl<sub>4</sub> (1.0 ml/kg, i.p., with olive oil, 1:1), at the doses of 100 and 200 mg/kg/day, p.o. for 7 days, significantly restored to all the serum and liver parameters near to the normal levels. The hepatoprotective potential of *S. oleracea* L. against hepatosuppression possibly involves mechanism related to its ability to block the P-450 mediated CCl<sub>4</sub> bioactivation through selective inhibitors of ROS (reactive oxygen species). Thus *S. oleracea* L., showing protection in liver, may prove as a rich source of antioxidants<sup>[44]</sup>.

### Inhibition of Clastogenesis

The homogenate of spinach reduces induction of micronuclei by benzo[*a*]pyrene (BaP) by 43–50% in the in vivo mouse bone marrow micronucleus assay. Inhibition of genotoxicity by spinach was not caused by any delay in

maturation of micro nucleated erythrocytes as shown by experiments with sampling times of 24, 48, and 72 h after dosing of BaP. Pre-treatment of the mice with spinach 48, 24, and 12 h before application of BaP resulted in a 44% reduction of micronuclei. A post-treatment procedure administering spinach 6 h after dosing of BaP did not indicate any protective effects. When *trans*-7,8-dihydroxy-7, 8-dihydrobenzo[*a*]pyrene (BaP-7,8-OH) was applied for induction of micronuclei spinach reduced the number of micronuclei by 55%. Pre-treatment of mice with spinach 96, 72, and 60 h before sacrifice caused a decline of hepatic 7-ethoxyresorufin-*O*-dealkylase (EROD) and of 7-pentoxoresorufin-*O*-dealkylase (PROD) activities by factors of 2.2 and 1.4, respectively. However, statistical significance was not reached<sup>[45]</sup>.

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### CNS Depressant Effect

Treatment with *Spinaciaoleracea* extract (SO; 400 mg/kg body weight) decreased the locomotor activity, grip strength, increased pentobarbitone induced sleeping time and also markedly altered

pentylenetetrazole induced seizure status in Holtzman strain adult male albino rats. SO increased serotonin level and decreased both norepinephrine and dopamine levels in cerebral cortex, cerebellum, caudate nucleus, midbrain and pons and medulla. Result suggests that SO exerts its CNS depressive effect in PTZ induced seizure by modulating the monoamines in different brain areas<sup>[47]</sup>.

### Inhibition of Proliferation of Human Gastric Adenocarcinoma Cells

Four kinds of assays (i) cell growth assay, (ii) colony forming assay, (iii) MTT colorimetric assay, and (iv) 3H-TdR incorporation assay, were used to study the effect of a fat-soluble extract of spinach powder (SPFE) on the proliferation of human gastric adenocarcinoma cell line (SGC-7901) in vitro. The concentrations of SPFE expressed as the level of beta-carotene in the medium were  $2 \times 10^{-8}$ ,  $2 \times 10^{-7}$  and  $2 \times 10^{-6}$  mol/L beta-carotene in assay (i)-(iii), but  $4 \times 10^{-8}$ ,  $4 \times 10^{-7}$  and  $4 \times 10^{-6}$  mol/L beta-carotene in assay (iv) respectively. The results indicated that SPFE inhibited the proliferation and colony forming ability of SGC-7901 cells. And in MTT assay, SPFE inhibited the viability of SGC-7901 cells, but no inhibitory effect of SPFE was observed on the viability of lymphocytes in peripheral blood of healthy people. Finally, in the 3H-TdR incorporation test, both SPFE and beta-carotene showed significant inhibitory effects on DNA synthesis in SGC-7901 cells, but SPFE was more effective than beta-carotene<sup>[48]</sup>.

### Anthelmintic Activity

Dave et al., 2009 evaluated the anthelmintic activity of crude extract of *Spinaciaoleracea*Linn. And different extract namely fresh juice extract and methanolic extract using *Pheretimaposthumaas* test worms. Different concentrations 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml of fresh juice extract and methanolic extract of *Spinaciaoleracea* Linn (MSO) were studied to determine the time of paralysis and time of death of worms. Both

the extract performed *invitro* anthelmintic activity. Albendazole was used as standard reference and saline water as control. The result was revealed that the fresh juice extract may show more potent anthelmintic activity than MSO<sup>[49]</sup>.

### CONCLUSION

Spinach (*Spinaciaoleracea*L.) is a leafy vegetable that belongs to the goosefoot family. Various pharmacological activities of *Spinaciaoleracea* such as, anti-oxidant, anti-inflammatory, antiproliferative, CNS depressant, antihistaminic, protection against gamma radiation, hepatoprotective have been reported. Various secondary metabolites like flavanoids, carotinoids, and phenolic compounds have been reported from this plant. Thus *Spinaciaoleracea* merits further phytochemical, pharmacological and clinical investigations for development of an effective natural remedy to provide therapeutically effective lead compounds or extracts.

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