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THE PHYTOPHARMACOLOGICAL REVIEW OF *BRASSICA NIGRA* SEED

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ABSTRACT

Brassica nigra are the species of Brassica genus (Family: Brassicaceae). The foregoing study showed tha*t Brassica nigra* possesses antioxidant, anti-inflammatory, antiepileptic, antidiabetic and many other pharmacological effects. This review will emphasize the chemical constituents and pharmacological effect of the plant.

KEYWORDS

Anti-oxidant, *Brassica nigra,* Pharmacology and Chemical constituents.

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INTRODUCTON

In prehistoric period Medicinal plants have been used for medicinal purposes. Ancient, Unani, Egyptian papyrus and Chinese writings described the use of herbs. Unani, Indian vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. India has been known to be rich receptacle of medicinal plants. More than 8000 herbal remedies have been categorized in AYUSH systems in India. Ayurveda, unani, Siddha and Folk medicines are the major systems of indigenous medicines. In worldwide 80% of people rely on herbal medicines for their primary health care needs. WHO states around 21,000 plant species have the potential for being used as medicinal plants. Medicinal plants are safe as there is no or minimal side effects. Many pharmaceutical

companies are conducting extensive research on plant materials to introduced new drugs in the medical practice[1]. Plants contain lot of secondary metabolites, which exerted a various pharmacological effects[2-57,1] *Brassica nigra* are species of Brassica genus (Family: Brassicaceae). This review will emphasize the phytochemical constituents and pharmacological effect of the plant.

Synonyms: Brassica nigra var. abysinnica A. Braun, Sinapis nigra L[58].

Taxonomic Classification[59,60].

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Family: Brassicaceae

Genus: Brassica

Species: *Brassica nigra*

Common names

Arabic: khardal aswad, khardal, Chinese: hei jie, English: black mustard, brown mustard, red mustard, French: moutard noire, Germany: Schwarzer Senf, Senf-Kohl, Japanese: kuro-garashi, Netherlands: zwarte Mosterd, Italy: Senape near, Philippines: mustasa; Portuguese: mostarda-negra; Sweden: svartsenap, Spanish: mostaza negra[59,60].

Distribution

Brassica nigra grows in temperate regions worldwide[61]. It is distributed in Albania, Bosnia, Algeria, Africa, ,Morocco, Eritrea, Tunisia, Libya, Asia, Armenia, Ethiopia, Afghanistan, Germany, Iran, Iraq, Lebanon, Turkey, Egypt, Kazakhstan, Europe, China, cybrus, Ireland, Belgium, United kingdom, Austria, Palastine, Czech Republic, Netherland, Hungary, Syria, Slovakia, Switzerland, Poland, Belarus, Moldova, Montenegro, Russian Federation, and Herzegovina, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, Denmark, Australia, Northern America, USA, Mexico, Canada, Southern America, Ecuador, Peru, Argentina and Chile, France, Norway, Spain, Newzeland[60].

Traditional uses

Brassica nigra used to treat rheumatism, as an agent to reduce congestion in internal organs. It was used in neuralgia and spasms, epilepsy, alopecia, snakebite, and toothache. Also used in the treatment of carcinoma and throat tumors. A seed liquid, when gargled, help tumors of the sinax. The seed was stimulate appetite and taken as a tonic. A bruised mustard seed with hot water makes a stimulating foot bath and also used as an inhaler, it completely cure cold or dispel a headache. Mustard oil stimulates hair growth and also used in hiccup. It was also considered as antiseptic[62]. Traditionally *Brassica nigra* is also used as simple rubefacient, diuretic, emetic, pneumonia, bronchitis, nerve stimulant and vesicant[63].

Description

Brassica nigra - Black mustard is an annual herbaceous plant. It grows up to 2 m, with many branches and the lower leaves are dentate, pinnatifid or lyrate and are often hairy. Upper leaves are narrow and oblong. In contrast many brassica species, the leaves are little it at all glaucous. The plants consists of yellow, four-parted and the cross-shaped flowers and produce 4 sided siliques capsular fruit that dehisces when mature that may be up to 2.5 cm long. Silique contains 2 to 12 or more reddish brown to black round seeds. One plant may produce thousands of seeds, which must be harvested by hand or mechanically before they fully ripen, because the siliques spontaneously split and disperse the seeds when they are mature[64].

Parts used

Seeds have more medicinal value from which oil is extracted[61].

Chemical constituents

Phytochemical studies showed that the plant contained alkaloids, flavonoids, glycosides, carbohydrates, sinapine, myrosin, sinigrin, inosite, albumins, gums, and coloring matters. The total phenol content in the plant was 6.67mg/g of gallic acid. It contained fatty oil (30-35%), Proteins (40%) Phenyl propane derivatives. Including sinapine and glucosinolates, chiefly sinigrin (allyl glucosinolates 1-5%). The seeds are grind into powder and mix with warm water releases the volatile mustard oil, allylisothiocynate[65-68].

The phenolic compounds gallic acid, quercetin, ferulic acid, caffeic acid and rutin were determined by HPTLC[69].

Hussien *et al*., reported that the callus of *Brassica nigra* was rich in secondary metabolites of volatile oils, anthraquinones, flavonoids and tannins. The calli obtained under light incubation conditions were higher in total phenolic than calli obtained under dark incubation conditions or the mother plant parts from which calli were induced[70].

Pharmacological effects

Antioxidant activity

The leaves were extracted with methanol and subjected to several in vitro studies. The methanolic extract of leaves was subjected to antioxidant activity with a concentration of 10-500µg/ml (1) and the antioxidant activity increased with the increase in concentration. HPTLC and GC-MS analyses carried out. The phenolic compounds gallic acid, followed by quercetin, ferulic acid; caffeic acid and rutin were determined by HPTLC. The antioxidant property of BN leaf extract suggests the presence of bioactive natural compounds[71].

Hepatic and Nephroprotective effect

Brassica nigra methanolic extract at doses of 200 and 400mg/kg body weight against D-GaLN (500mg/kg body weight) induced toxicity, with silymarin used as the standard. Tissue damage, activities of serum marker enzyme, hematological changes, metabolites such as bilirubin, urea, uric acid, and creatinine levels, tissue thiobarbituric acid reactive substance, non-enzymic and enzymic anti-oxidants and inflammatory marker enzymes such as cathepsin D, myeloperoxidase, and acid phosphatase were assessed[72].

The toxicity induced by the D-GaLN was evident from a significant increase ($p< 0.001$) in the serum and tissue inflammatory markers in toxic rats, when compared with the control (saline alone treated animals). The pretreated groups with *Brassica nigra* (200mg and 400mg/kg body weight) showed significant ($p< 0.001$) reduction in the D-GaLN-induced toxicity as obvious from biochemical parameters. Histopathological studies confirm the protective effect of *B.nigra* leaf extract by reduction in hepatic and renal tissue damage. Experimental extract showed similar result as the standard. Leaf extract of *Brassica nigra* exhibit hepatic and nephroprotective effects against D-GaLN- induced toxicity in Wistar rats.

Antimicrobial activity

Leaves of *Brassica nigra* were extracted by continuous hot extraction and different concentrations were obtained by ethanol, n-hexane, aqueous and DMSO solvents. Microorganisms (Staphylococcus aureus and Escherichia coli from bacterial strains and Candida albicans from fungal strain) were selected for testing antimicrobial activity of the plants. Then the extracted solutions were diffused to selected standard organisms inoculated in Muller Hinton Agar using well diffusion technique. *S.alba* ethanolic extract of 2500mg/ml dissolved in DMSO concentration against E.coli have shown a significant activity with inhibition zones of 30mm. Also had a considerable effect in same concentration against *S.aureus* and *C.Albicans* with a prompting result of 28mm and 25mm zones of inhibition respectively which is greater than the positive control. This plant showed an equal activity at 1000mg, and 250mg which are 20mm and 13mm respectively for *C.Albicans,* 26mm and 23mm for *S.aureus* and for *E.coli* 25mm and 17mm. N-hexane extract of the same plant also showed a remarkable activity at concentrations of 1000mg, 250mg and 50mg, where the zones of inhibition against *S.aureus* were 18mm, 20mm and 25mm respectively. Ethanolic extract of *Brass nigra* leaves showed activity at the lowest concentration. N-hexane and Ethanol extracts gave a remarkable activity against all the selected microorganisms[73].

Antibacterial activity of oils extracts of *Brassica nigra* seeds on some bacteria isolated from plaque and healthy teeth in children (1-5) years

In this study, from dental plaque and healthy teeth (control) some bacterial species were isolated and identified in children (1-5 years) that included pathogenic and non pathogenic staphylococci, streptococcus spp. Escherichia coli and proteus spp. Also they studied the antibacterial activity of oils of extracts from *Brassica nigra* seeds and some antibiotics by disk diffusion methods. Results showed that the isolates were more sensitive to oils alcoholic extracts compared with aqueous extracts

and they were more sensitive to ciprofloxacin and Gentamicin compared with other antibiotics[70].

Evaluation of Antimalarial activity of 80% methanolic extract of *brassica nigra* (L) Koch. (Brassicaceae) seeds against *Plasmodium berghei* infection in mice

To evaluate the antimalarial activity of 80% methanolic extract of the seeds of *Brassica nigra* against Plasmodium berghei infection in mice. Plasmodium berghei was Chloroquine sensitive (ANKA strain) so it was used to test the antimalarial activity of the extract. In suppressive and prophylactic models, swiss albino male mice were randomly grouped into five and each group consists of five mice. Control Group I mice were treated with the vehicle, group II, III, IV were treated with 100, 200 and 400mg/kg of the extract, respectively and the last group (v) mice were treated with chloroquine (10mg/kg). The level of parasitemia, survival time and variation in weight of mice were used to determine the antimalarial activity of the extract[74].

The extract of the seeds of *Brassica nigra* exhibited Chemo suppressive activities were 21.88, 50.00 ($P<0.01$) and 53.13 % ($P < 0.01$) while chemo prophylactic activities were17.42, 21.21 and 53.79% ($P<0.05$) at 100, 200 and 400mg/kg of the extract respectively as compared to the negative control. Mice treated with 200 and 400mg extract were significantly ($P<0.05$) lived longer and gained weight as compared to negative control in 4-day.

Antioxidant and anti-inflammatory activities of the leaf extract of *Brassica nigra*

Brassica nigra ethanolic extract was studied to detect the chemical compounds as well as to evaluate the antioxidant and anti-inflammatory activities. The different antioxidant assays including total antioxidant activity, DPPH, nitric oxide (NO) scavenging, reducing power, total phenolic content and flavonoid content were studied. Phytochemical studies showed the presence of alkaloids, flavonoids, glycosides and carbohydrates in the extract. Total phenol content in the plant was 6.67mg/g of gallic acid. *Brassica nigra* was found to contain 2.04mg/g of quercetin in flavonoid assay. Total antioxidant capacity of extract was found to be 97.08mg/g of ascorbic acid. *Brassica nigra* showed IC50 value of 63.09 whereas standard antioxidant showed IC50 value 14.45µg/ml in DPPH method. The standard antioxidant ascorbic acid, gallic acid, and quercetin showed the reducing power 485.75%, 736.30% and 763.01, respectively whereas *Brassica nigra* showed the value 263.69%. IC50 value in NO scavenging activity of the extract was found to be 118.21µg/ml whereas ascorbic acid showed the value 5.47µg/ml and quercetin had the value15.24µg/ml. *In vivo* and in vitro anti-inflammatory activity of the crude extract was evaluated using carrageenan induced rat paw edema and protease enzyme inhibition assay respectively. *In vivo* anti-inflammatory test of the ethanolic extract of *Brassica nigra* (500mg/kg) gave 17.9% inhibition whereas standard phenylbutazone (100 mg/kg) gave 39.38%. *In vitro* anti-inflammatory test of *B.nigra* by protease inhibition method also gave 42.57% inhibition of trypsin at dose 250µg/ml[75].

Antiepileptic effect

Antiepileptic activity of methanolic extract of *Brassica nigra* seeds was investigated on maximal electroshock-induced seizures (MES), Pentylenetetrazole (PTZ), Picrotoxin (PIC) and biccuculine induced seizures in mice. It was found that the extract (200 and 400 mg/kg, orally), significantly prolonged the onset of tonic seizures and reduced the duration of incidence of seizures in PTZ, PIC and biccuculine induced seizure models, in MES model, the extract showed significant effect in abolishing tonic hind limb extensions by inhibiting voltage dependent Na^+ channels or by blocking glutaminergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor[66].

The anticonvulsant effect of the methanolic extract of *Brassica nigra* seeds (75, 150 and 300mg/kg; ip) was evaluated in pentylenetetrazole (PTZ) - induced kindling in mice. The methanolic extract of *Brassica nigra* seed reduced the intensity and duration of seizure. The *Brassica nigra* extract increased the SOD and NO levels and decreased the MDA level in the brain tissues[71].

Antidiabetic effect

The diabetic was induced in rats with Streptozotocin followed that they were treated with

aqueous, ethanol, acetone and chloroform extracts of the seeds of *Brassica nigra,* the increase in serum glucose value between 0 and 1 hr of glucose tolerance test (GTT) was the least (29mg/dl) in aqueous extract treated animals, and it was 54, 44 and 44mg/dl with chloroform, acetone and ethanol extracts respectively. The effective dose of aqueous extract was found to be 200mg/kg body weight in GTT. Aqueous extract was administered to diabetic animals (200mg/kg body weight) once daily for one month brought down fasting serum glucose (FSG) levels. The Hb A1 c and serum lipids in the treated group were much less than untreated diabetic controls[72].

Brassica nigra aqueous extract has been shown to have good antidiabetic effect along with significant decrease ($p<0.01$) of abnormal serum lipid levels. The mechanism of this effect was studied via investigation the effect of oral administration of AEBN for two months on glycolytic and gluconeogenic enzymes in liver and kidney tissues of rats with streptozotocin (STZ) induced diabetes mellitus. The gluconeogenic enzymes activities were higher and of glycolytic enzymes were decreased in both the liver and kidney tissues during diabetes. However in diabetic rats treated with AEBN for two months, decrease of serum glucose, increase of serum insulin and release of insulin from pancreas (shown *in vitro* isolated from pancreas) along with the restoration of key regulatory enzyme activities of carbohydrate metabolism and glycogen content were observed. The therapeutic role of AEBN in STZ induced diabetes can be attributed to the release of insulin from pancreas and change of glucose metabolizing enzyme activities to normal levels, thus stabilizing glucose homeostasis in the liver and kidney. The LD 50 was found to be more than 15 times the effective dose (ED) implying higher margin of safety for AEBN[63].

Other effects

Mustard is a stimulating condiment and appetizer, and excites gastric activity and promotes digestion. If the amount be large, however, it is intense irritation and promptly causes vomiting[76]. Alcoholic extracts of seeds of *Brassica nigra Linn* was investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*[77]. The effects of various concentrations (10-100 mg/ml) of extract were tested on the paralysis and time of death of the worms. Alcoholic extracts exhibited significant activity at high concentration (100mg/ml).

The local action of mustard may stimulate the cardiac and respiratory activity in sufficient force to arouse one from an attack of fainting. Both the breathing and circulation are stimulated by its reflex action upon the respiratory centers and the heart[76].

Contraindications and adverse effects

The methanolic extract of *brasssica nigra* seeds was administered orally to different groups of mice at different dose levels and found to be safe and did not produce any mortality or toxic symptoms even up to the dose level of 2000mg/kg[66,67]. However, the use of black mustard is contraindicated in individuals with gastrointestinal ulcers or inflammatory kidney diseases and was not used for children under 6 years. Hyperthyroidism with goiter occurred with the use of the isothiocynates in mustard. Avoid taking with ammonia containing products because ammonia with mustard oil yields inactive thiosinamine. Mustard oil is absorbed through the skin. Even external poultice should be limited to 5-10 minutes pediatrically, 10-15 minutes for adults less for sensitive patients. 15-30 minutes plaster can cause severe burns. It caused skin and nervous damage in prolonged use, should not be used for more than 2 weeks. Gastrointestinal complaints (and rarely kidney irritation) could occur following internal administration, due to mucus membrane irritating effect of the mustard oil. The drug possesses minimal potential for sensitization, contact allergies have been observed. The draining effect associated with the drug's administration makes it inadvisable in the presence of varicose and venous disorder. Sneezing, coughing and possible asthmatic attacks can result from breathing the allylisothiocyanate that arises with the preparation of and application of mustard poultices. Eyes should be protected when preparing or using the poultices because the vapors can cause eye irritation. Long-term external application or too-intensive reactions upon the skin can lead to injury such as blister

formation, suppurating ulcerations and necroses. Mustard oil poultices are to be removed after not more than 30 minutes[58-61].

Dosage

Used externally as a mustard plaster, foot bath or full bath. The preparation is placed on the skin for about 10 minutes (with a maximum of 3 to 5 minutes for children more than 6 years). Foot bath use should be limited to 10 minutes. On rare occasions, Black mustard is used as a constituent in antirheumatic preparations[61].

Brassica nigra

CONCLUSION

The previous studies showed that *Brassica nigra* possessed antioxidant, anti-inflammatory, antiepileptic, antidiabetic, antibacterial, antimalarial, antimicrobial, Hepatic and nephroprotective activity and anthelmintic activity. This review will highlight the chemical constituents and pharmacological effect of the plant.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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