Potent antioxidant, anti inflammatory activity and calcium channel blocker effect of Solanum nigrum in animal model

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ABSTRACT

Throughout history,S. nigrum has been used to treat a wide range of diseases, such as pain, irritability, and fever. For a range of medical purposes, including as an antioxidant, antitumorigenic, anti-inflammatory, hepatoprotective, diuretic, and pain reliever, Eastern civilizations also employ this herb. There are numerous compounds known to control a wide range of processes. The results show that S. nigrum extracts from the aerial component may be used as anti-inflammatory agents. In this paper, we investigated the maethanolic extract of solanum nigrum's antioxidant, anti-inflammatory, and calcium channel blocker properties. Solanum nigrum extract from berries and aerial parts is used in various concentrations. Solanum nigrum aerial parts and berries were tested for their ability to scavenge DPPH free radicals. In the end, it was determined that berry extract had higher antioxidant levels than aerial component extract.

KEY WORDS

Black nightshade, Skin problems, Dropsy, Swelling of joints, Osteoporosis, Pain revealing , Therapeutic plant

1.Introduction

Due to the growing problem of disease resistance, finding novel and potent antiinflammatory and antioxident medicines is one of the major difficulties facing modern medicine and agriculture. [1] Despite the widespread use of synthetic care products and plant protection goods in this period of rapid technological advancement, natural ingredients continue to enjoy unwavering appeal [2] . A member of the Solanaceae family native to Europe, Asia, and North America, Solanum nigrum L. (SN) was also brought to South America, Australia, and Africa. It represents one of the genus's largest and most diverse species groups [3] . Black nightshade, also known as Solanum nigrum, is a well-known herbal remedy. Researchers have examined the well-known Solanaceae family for its potential medicinal properties [4] , are widely known for their therapeutic benefits. S. nigrum has been utilized historically, to treat a variety of illnesses include discomfort, irritation, and fever.[5] Pakistan is expected to have 450,000 people who are ill with serious diseases.[6] Eastern cultures also make use of this herb systems of healthcare for a variety of reasons, including antioxidant, antitumorigenic anti-inflammatory, hepatoprotective, diuretic, and a painkiller. Numerous substances have been recognized that are in charge of a variety of operations.[7]. According to research, the Solanum nigrum plant has about 188 various chemical parts that may be distinguished from one another. The four most important bioactive elements found in these chemical substances are referred to as "steroidal saponins," "alkaloids," "phenols," and "polysaccharides," in decreasing order of value [8] .

According to reports, calcium channel blockers can reduce excessive urination, which reduces the body's water content and eases kidney stone symptoms [9]. When combined with other medications, calcium channel blockers, which widen blood vessels by limiting calcium flow into cells, efficiently lower blood pressure.[10] Some formulations of these medications are also licenced for the treatment of angina or cardiac dysrhythmias. [11] For patients with cardiac dysrhythmias or who require blockers, nondihydropyridine calcium channel blockers are more adversely chronotropic and inotropic than the dihydropyridine subclass.[12] Calcium channel blockers (CCBs) prevent the movement of extracellular calcium through cell wall-spanning ion-

specific channels.[13] Although there are various kinds of these channels, the l-type channels in people are inhibited by the CCBs that are now on the market. Vascular smooth muscle cells relax as a result of a reduction in inward calcium flow, which lowers blood pressure (BP) and causes vasodilation [14]. Contractility is decreased, and sinus pacemaker and atrioventricular conduction velocities are retarded in the heart muscle [15]

Additionally, they are employed in the management of heart and blood circulation issues [16] . Additionally, the use of corticosteroid calming medications has been limited because they were linked to weight gain and osteoporosis. While NSAIDs taken at high doses can cause gastrointestinal tract toxicity [17] . The entire plant of Solanum nigrum L. can be utilised for the experiment to test the anti-inflammatory properties. For the experiment to evaluate the anti-inflammatory effects in rats, the extract of the entire plant is employed. And their effects are contrasted with those of common medications like indomethacin and cyproheptadine, which are administered in varying doses. Thus, the findings support the traditional usage of Solanum nigrum as an anti-inflammatory agent [18] . In China, experiments have shown and verified that the plant inhibits or prevents the growth of cervical cancer [19] . Scientists are looking for anti-HCV plants all throughout Pakistan to treat this illness, and these plants don't have any negative side effects like the interferon that was once used. Solanum nigrum seed extracts in methanol and chloroform show anti-HCV properties of 35% and 50%, respectively. Additionally, these don't harm the patients in any way. The following proposal is that this can be used to cure hepatitis [19] . Using several cell lines, Hepatome (HepG2) and Ehrlich ascites cell (EACC) lines. The active component of solanum nigrum was evaluated for its ability to suppress cancer. The 2, 2 diphenyldipicryl hydrazile (DPPH) technique is used to assess the anti-oxidant activity. When the chemicals are well characterised, their antioxidant values are modifiable. Thus, it may be concluded from the study that a variety of substances derived from Solanum nigrum's ethanolic extract can be utilised to treat cancer, a serious illness that often results in patient death [20]. Application of Withania coagulans was made to rabbit jejunum preparations to test for potential calcium channel blocking properties.[21] Different dosages of the extract of methanol were employed.The tissues provide dosage response curves and confirm the calcium channel blocking activity of tyrode's calcium-free solution. Finally, the outcomes supported the plant extract's ability to inhibit calcium channels [22].

2.Material and Methods

2.1 Materials

Methanol , Distilled water, Conical flasks cleaned with detergent, oven for drying, powdered berries, Aluminium paper, filterpaper, air tight bottles, rotatory evaporator, vortex mixer, DPPH solution, nifedipine, cyanide-3-glucoside

2.2 Methods

2.2.1 Collection of plant material

The Solanum nigrum plant material was gathered from a university allotment. It was cleansed twice or three times with tap water. It was kept in the shade until it had fully dried. Put it through a grinder, then dip it in methanol to continue the process.

2.2.2 Preparation of Solanum nigrum methanolic extract

Plant material was dried separately in the shade It was checked to make sure there were no impurities, such as dust and other undesirable stuff. Then, it was ground in a grinder to create a homogenous powder that contained the aerial and berry components separately. Conical flasks were cleaned with detergent, followed by a methanol rinse. Make sure to thoroughly rinse the conical flasks with methanol before drying them in the oven. Following the correct drying of the flasks, 200 mg of powdered berries and aerial portions were taken in separate conical flasks. After that, enough methanol was added to soak the powder completely. Methanol was even layered an additional 1 cm thick. Additionally, none of the powder's particles float on this methanol layer. After that, aluminium paper was placed over the flasks' mouths. On rare occasions, these flasks were shaken for a day before being filtered via filter paper. In order to control the float and ensure that no soaking powder leaks out, the mouth of conical flasks was covered with four layers of gauge. Filtrate is consumed separately in airtight bottles. For three days, this technique was done three times. These were evaporated in a rotary evaporator at 35°C after being filtrated. After the filtrate had completely evaporated, hard material was produced. The flask is then filled with methanol, which is added and mixed in a vortex mixer. It was confirmed that there were no further residues on the flask wall. We placed it in a container covered with aluminium paper, poured

methanol into the container, and the methanol evaporated, yielding a sticky methanolic extract of Solanum nigrum.

2.2.3 The extract solution's preparation to use for the experiment

When the plant extract was fully dried, it was weighed, made by dissolving in sterile, distilled water. Weighed and dissolved in sterile distilled water, the dried plant extracts were diluted to the necessary amounts of approximately 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml, and 2.5 mg/ml. Unless they were being utilised in the experiment, they were kept in the refrigerator.

2.2.4 Antioxidant Activity of Solanum nigrum

Various concentrations of Solanum nigrum extract were diluted (200 g/ml, 400 g/ml, 600 g/ml, and 800 g/ml) **.**To make the DPPH solution, 10 mg of DPPH was dissolved in 100 ml of methanol.1 ml of the extract from each dilution was added to the test tube's 2 ml of DPPH solution.2 ml of DPPH solution were combined with 1 ml of methanol. After vigorous shaking, the mixture was kept in the dark for 30 minutes. The solutions were computed using spectrophotometry at 517 nm to determine their absorbance.

Following were the formulas used to determine each extract's DPPH radical scavenging capacity.

Equation: % scavenging DPPH free radical = 100 × (1-AE/AD)

Where

AE= Absorbance of the solution

AD = Absorbance of the DPPH solution without extract (Control)

2.3 Solanum nigrum anti inflammatory activity

Five groups of 200–250 g, both male and female rats were used. Six rats are in each group. Group 1 served as the control group and was given a suspension of CMC (carboxyl methyl cellulose). Cyanidine-3-glucoside was administered to Group 2. Group 3 received 125 mg/kg of the Solanum nigrum berry extract. Group 4 got a concentrated (375 mg/kg) preparation of the aerial portions of Solanum nigrum. Diclofenac was given to Group 5. 0.1 ml of carrageen in saline was administered into the left hind paw after an hour of therapy to induce paw edoema. After injecting carrageenan for 0, 1, 2, and 3 hours, the paw volume was measured.

2.4 In vitro Ca2+ antagonistic activity of Solanum nigrum

We utilised Sprague Dawley rats. Rat aorta strips that were spirally sliced and 7 cm long were utilised. Rat aorta in isolation was kept in a depolarizing solution. CaCl2 was utilised in the organ bath as a control. It demonstrated consistent responses to Ca2+ in 30 cc of Krebs nutrient-filled organ bath. Oxygenated and carbon dioxide-filled. A 90-minute stabilising time was required. A depolarizing solution was then used in place of the Krebs solution. It caused the aorta to constrict in preparation before relaxing it. The agonists employed were nifedipine, cyanide-3-glucoside, and extracts of the berries and aerial portions of Solanum nigrum. An agonist was created using nifedipine, cyanide-3-glucoside, and extracts from berries and the aerial portions of Solanum nigrum.

3. Results

The anti-oxidant activity of Solanum nigrum was assessed using DPPH free radical scavenging. This was established using varied doses of leaf and berry extract (200 g/ml, 400 g/ml, 600 g/ml, and 800 g/ml). As a control, a DPPH solution without extract was employed. The % inhibition of berry and stem methanol extracts was calculated (Table 1). The percentage inhibition rises as the concentration rises. Therefore, in both situations, the concentration of 80 g/ml results in the maximum percentage of extract inhibition. Berries' methanolic extract shown greater antioxidant activity than the extract from the aerial component. Therefore, it is evident that berry extract has a strong potential for becoming a potent anti-oxidant.

Table 1. The DPPH free radical scavenging activity of barriers and Aerial parts of S.Nigrum

Concentration µg/mL	Methanol extract of berries	Methanol extract of aerial parts
200	63.02	60.11
400	65.04	61
600	72.05	69.72
800	76.06	72.22

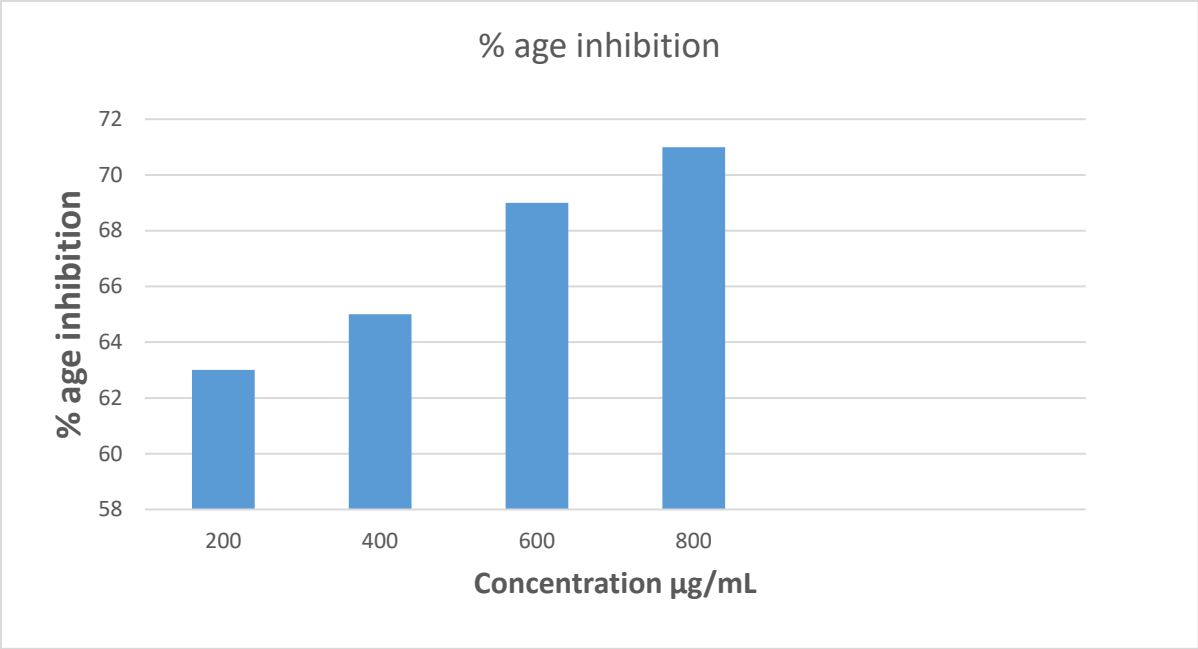

Figure 1. DPPH free radical scavenging g activity of Berries of Solanum Nigrum

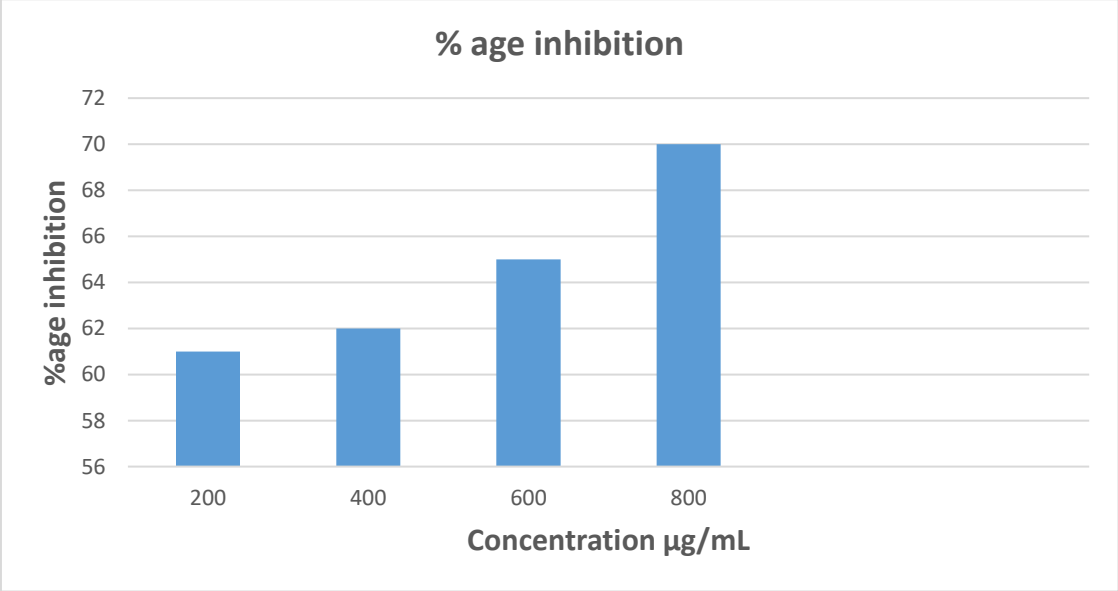

Figure 2. DPPH free radical scavenging activity of Aerial parts of Solanum nigrum

The effect of Solanum nigrum's aerial and berry parts' methanolic extract shows notable results in anti-inflammatory activities shown in Table no.2. Carrageenan in ordinary saline caused inflammation to be induced in the paws of rats. Their results were contrasted with a control group that received carboxymethylcellulose (CMC) suspension in saline. Additionally, cyanidine-3-glucoside exhibits anti-inflammatory properties. Significant 3-glicoside inhibition is seen in the aerial portions of Solanum nigrum. Following three hours, berries extract and cyanidine-3-glucoside reduced the edoema by 25%.

Sr. No.			Odema Diameter (cm)	Odema Diameter (cm)	Odema Diameter (cm)	Odema Diameter (cm)
1	Name of Samples	Dosage (mg/kg)	0 hr	1 hr	2 hr	3 hr
2	Control (Normal Saline) 10ml/kg	Sham	0.96 ± 0.002	0.98±0.003	0.98 ±0.003	1.01 ±0.02
3	Cyanidine-3-glucoside	120	0.91 ±0.008	0.86±0.004	0.84 ±0.003	0.81 ±0.005
4	S.nigrum Berries	125	0.86 ± 0.008	0.84 ±0.01	0.83 ±0.008	0.81 ±0.003
5	S.nigrum Aerial parts	375	0.90 ± 0.008	0.76±0.003	0.77 ±0.006	0.75 ±0,01
	Diclofenac	10	0.93 ± 0.003	0.90±0.003	0.89 ±0.003	0.86 ±0.05

The complete results of the in vitro study that was conducted on an isolated rat aorta are provided in Table 3, which summarises the findings of this investigation. The competition of antagonistic responses of other test compounds in the presence of various concentrations of CaCl2, which is utilised as the control, led to pA2 estimations. According to the results, the compounds examined can be grouped as nifedipine, cynidine-3-glucoside, extract of Solanum nigrum berries, and extract of Solanum nigrum aerial parts, in descending order of their activity. According to these findings,

Solanum nigrum extract from the aerial parts has the lowest activity compared to other extracts, whereas Solanum nigrum extract from the berries has a higher level of activity. Cynidine-3-glucoside is more active than Solanum nigrum extracts but less active than nifedipine.

Table 3. pA 2 of the substances under study as calcium antagonists in the isolated rat aorta

Substance	Concentration	pA2
	[M]	
Nifedipine	2.78×10^{-9}	2.76
Cynidine-3-glucoside	4.07×10^{-6}	2.05
S.nigrum berries	5.52×10^{-5}	1.88
S.nigrum Aerial parts	7.49×10^{-5}	1.76

4.DISCUSSION

Due to the toxic effects of the present therapy used to treat those inflammation using synthetic pharmaceuticals, herbal remedies are becoming increasingly important in the treatment of inflammation. When compared to synthetic pharmaceuticals, herbal medicines are less expensive and hazardous.[23] The current study will assist the sector in producing an herbal medicine that is less harmful, more inexpensive, and effective at treating inflammation [24] There are two types of Solanum nigrum: one has fruit that is black in colour and the other is reddish brown. Fruit with a black tint is poisonous in both types.For health purposes, the entire plant, the roots, and the leaves are employed [25] The extract of S. nigrum demonstrated antinociceptive, anti-inflammatory, and antipyretic effects in the current study when evaluated utilising several animal models [26]. In traditional Ethiopian medicine, the plant Solanum nigrum L. is used to treat malaria. The objective of this work was to assess the antimalarial efficacy of crude extract and fractions from S. nigrum L. (Solanaceae) leaves against P. berghei infections in mice. [27] Overall, methanol extracts beat Solanum nigrum aqueous leaf extract in terms of effectiveness.[28] . The leaves can provide significant amounts of fat, fibre, vitamins A and C, calcium, iron, and phosphorus, as well as protein and amino acids. The berries also appear to contain significant amounts of vitamin C and beta-carotene, as well as high levels of iron, calcium, and vitamin B. The seeds also include

carotene and vitamin C [29] . The outcomes of methanolic extract are comparable as well. When treating the irritation with natural remedies, they also used carrageenan and methanolic extract. Inflammation so drops by 48%. Carrageenan, which causes paw edoema, and the berry extract in methanol were both studied in the same manner. Additionally, this methanolic extract reduced the edoema that developed in the back paw [30] . Additionally, consuming nightshades may cause respiratory, cardiovascular, and gastrointestinal problems (such as diarrhoea, nausea, or vomiting). However, black nightshade was still utilised in conventional medicine to treat wounds, ulcers, diarrhoea, as well as toothaches and nosebleeds [31]. S. nigrum has been widely grown in China in recent years because its unripe fruit may be put into hospital preparations for adjuvant therapy of various tumours and its ripe fruit can be processed into food products. [32] Significantly anti-inflammatory effect has been demonstrated by Solanum nigrum extract at a dose of (375 mg/kg).While the concentration utilised in the current study (125 mg/kg) produced significant outcomes, such as a 20% reduction in edoema after 3 hours. Therefore, the comparison significantly supports the anti-inflammatory efficacy of Solanum nigrum in the current investigation. Additionally, it has been noted that there is a strong relationship between antioxidant chemicals and calcium channel blockers (CCBs).[33] Because calcium can produce contractions in depolarized smooth muscles, calcium channel blockers (CCBs) were previously known as calcium antagonists [34] . CCBs have traditionally been used to treat heart disease and hypertension.[35] Stopping the entry of calcium reduces the vascular smooth muscle's active propensity and causes vasodilatation. [36] Mostly the calcium channel blocker subtype of dihydropyridine. In comparison to other kinds of antihypertensive medications such diuretics, angiotensin-converting enzyme (ACE) inhibitors, and beta-blockers, it was more effective at reducing carotid intima-media thickness (IMT). [37] A study found that CCBs that are lipophilic, such lacidipine, may reduce oxidative stress and ox-LDL-induced propagation in vascular smooth muscle cells. Additionally, these calcium channel blockers are utilised as a blood pressure and diabetes medication [38] . The limitations of research on medicinal plants are reviewed in order to address the growing demand for safer and more potent epilepsy medications [39] . Some of the plants, including Piper longum, Ocimum tenuiflorum, and Solanum nigrum, are effective in treating allergic illnesses of the respiratory system [40].

CONCLUSION

This study examines the methanolic extract of Solanum nigrum's antioxidant, anti-inflammatory, and calcium channel blocker properties. Using methanol, dried berries, and aerial portions were removed. In comparison to the extract of aerial parts, the berry extract contained higher antioxidants. Rats also displayed anti-inflammatory effects when exposed to Solanum nigrum methanolic extract. They are given carrageenan to make them swollen, and the methanolic extract of Solanum nigrum significantly reduces inflammation. The extract was additionally employed to test the rat aorta's ability to inhibit calcium channels. The following compounds can be grouped according to how well they block calcium channels, in descending order of activity: Nifedipine, cynidine-3-glucoside, Solanum nigrum berry and aerial component extracts, and nifedipine. Thus, it was determined that Solanum nigrum considerably exhibited anti-oxidant, anti-inflammatory, and calcium channel blocking action.

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