Anti-inflammatory, antinociceptive and antioxidant activities of ethanolic extract of *Solanum nigrum L.* fruits

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Abstract

Ethanolic maceration extract of *Solanum nigrum L.* fruits belonging to solanaceae family was used. Anti-inflammatory activity of the extract was examined using carrageenan induced paw edema and xylene induced ear edema in mice. Antinociceptive activity was studied using the hot plate and writhing tests. The study was carried out using doses of 100, 200 and 400 mg/kg orally. The extract showed significant anti-inflammatory and antinociceptive activity at the dose of 400 mg/kg ($P < 0.01$) as compared to standard drug Diclofenac sodium (50 mg/kg). Antioxidant and reducing power activities were evaluated using different concentration (100-400 µg/mL) of ethanol extract. The extract had the most potent antioxidant effect toward linoleic acid model system, and strong reducing power ability.

Key words: *Solanum nigrum*, anti-inflammatory, antinociceptive, antioxidant properties.

الخلاصة

درست فعالية المستخلص الايثانولي لمنقوع نبات عنب الذيب كمضاد للالتهابات وكمسكن للآلام على ألجرذان ألمختبريه، وذلك بتجريعها فمويا تراكيز مختلفة 100، 400،200ملغم/كغم من ذلك المستخلص. أظهرت ألدراسة فعاليه معنوية عند الجرعة العالية 400ملغم/كغم وبفارق معنوي ($P < 0.01$) مقارنة مع عقار معروف هو صوديوم دكلوفيناك (50 ملغم/كغم). كما تم دراسة الفعالية المضادة للأكسدة للمستخلص الأيثانولي وذلك بأخذ تراكيز مختلفة تراوحت بين (100-400 مايكروغرام/مل). إذ أظهر المستخلص فعالية مضادة لأكسدة حامض اللينولينك وقابلية اختزال قويه في النظام النموذجي .

Introduction

It is known that a large number of plant species contain various bioactive compounds that may have health-beneficial properties, anti-inflammatory, anti-oxidant and antimicrobial effects, and their preventive and therapeutic use is increasing [(1)]. Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [(2)], currently available analgesic drugs such as opiates and NSAIDS are not useful in all cases due to their adverse effects [(3)]. Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain and inflammation [(4)]. Moreover, a number of pathologies are known to be ultimately associated with the imbalance of pro- and antioxidant factors in living systems. Exogenous antioxidant compounds may therefore exert beneficial action upon systems which have been deprived from sufficient amounts of endogenous antioxidants as in some cardiovascular diseases, tumors, inflammation, ulcer and aging [(5)]. Currently, the possible toxicity of synthetic antioxidants has been criticized. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years [(6)]. *Solanum nigrum L.* (Solanacea) is a thorny shrub widely distributed in moist places of Iraq. This plant is well known in English and Tamil system as ' Black night shade ' and ' Kakamachi ', respectively [(7)]. This research was aimed to investigating the possible anti-inflammatory, anti-neociceptive and antioxidant activities of *S. nigrum* fruits.

Materials and Methods

Chemicals

The following chemicals have been supplied by Sigma and Aldrich Co. (St. Louis, MO) **:** Thiocyanate, Potassium ferricyanide, Trichloroacetic acid Tween 20, KH_2PO_4, Na_2HPO_4 and $FeCl_3$, Carrageenan**.** E. Merck (Darmstadt, Germany) supplied the following chemicals **:** Ethanol, acetic acid and linoleic acid**.** All of the reagents were prepared in deionized distilled water (ddH_2O) to eliminate the contamination of metal ions.

Plant Material and Extraction procedure

Fruits of *Solanum nigrum L.* were collected from the garden of Science College, University of Basrah in the month of April, 2009 and the plant was botanically authenticated and voucher specimens 3899 were deposited in the Herbarium of Basrah (Iraq, Basrah, College of Science, University of Basrah). The shade-dried powders of fruits extracted in a soxhlet apparatus with 80% ethanol gave 7.21g of dry extract.

Animals

Inbred colony of adult male and female Swiss albino mice (20-35g) of either sex were used for both anti-inflammatory and antinociceptive activity. They were housed in polypropylene cages under a 12 h light**:**12 h dark cycle in a controlled temperature room (25±2 °C). All the animals were acclimatized to the laboratory conditions for a week before use. They had free access to food and water. All studies were carried out by using five groups of six animals (3 males and 3 females), as follow:

Group I served as control received 2% w/v Tween 80 in normal slain, 10mL/kg (P.O), Group II received Diclofenac sodium, 10mg/kg (P.O),

Group III, IV and V received ethanolic extract (100, 200 and 400 mg/kg (P.O) of *S. nigrum* respectively.

Anti-inflammtory study

Carrageenan-induced paw edema

According to the technique of Winer *et al*.[8] determined the carrageenan-induced paw edema. After 0.5 h, 0.1mL of 1%w/v carrageenan suspension was injected subcutaneously to the plantar surface of the left hind paw. The paw volume was measured using a plethysmometer (model 7140, Ugo Basile, Italy), immediately and 0.5, 1, 1.5 and 2h after drug treatment. The anti-inflammatory effect is expressed as the average percent inhibition of edema, which is calculated by the following equation:

% inhibition = [1 – Vt /Vc] × 100

Where Vt and Vc represent the increase in paw volumes of mice treated with drug and control, respectively.

Xylene-induced ear edema

A published method by Hosseinzadeh *et al*. [9] was adopted. After 0.5h, 0.03mL xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. Two hours after xylene application, mice were killd and both ears were removed. Circular sections were taken, using a cork borer with a diameter of 7mm, and weighed. The increase in weight caused by the irritant was measured subtracting the weigh of the untreated left ear section from that of the treated right ear sections. The anti-inflammatory effect is expressed as the average percent inhibition of writhes, which is calculated by the following equation:

% inhibition = [1 – Vt /Vc] × 100

Where Vt and Vc represent the average writhes in the drug and control groups, respectively.

Antinociceptive study

Hot-plate test

The hot plate test was assessed according to the method described by Hosseinzadeh *et al*.[9], with minor modification. The temperature of a metal surface was maintained at 55±0.5°C. Latency to a discomfort reaction (jumping, withdrawal or licking of the paws) was determined befor and after drug administration. A cut-off time was 15 sec, to avoid damage of the paw. Reaction time and the type of response were noted using a stopwatch. The latency was recorded befor and 0.5, 1, 1.5 and 2 h after oral administration of both test and standard drugs. Average reaction times were then calculated and the percentage variation calculated using following relation:

% inhibition = [(Before treatment / After treatment) – 1] × 100

Writhing test

The test was performed as described by Ahmed *et al*. [4]. Test, standard samples and control were administred orally 30 min befor intraperitoneal administration of 0.7% v/v acetic acid (volume of injection 0.1mL/10g body weight). The mice were placed individually into glass beakers and 5min were allowed to elapse. The number of stretching or writhing was recorded for the next 10 min.

Antioxidant activity study

Linoleic acid-system

This test was carried out by using the method of Snedecor and Cochran[10]. Linoleic acid emulsion (0.02 M) was prepared with linoleic acid (0.2804 mg) and Tween 20 (0.2804 g) in phosphate buffer (50 mL, 0.05M, pH 7.4). A reaction solution, containing extract 100-400 µg/mL. Linoleic acid emulsion (2.5mL) and phosphate buffer (2.3 mL, 0.2 M, pH 7.0) were mixed with a homogenizer. The reaction mixture was incubated at

37°C in the dark and the degree of oxidation was measured according to the thiocyanate method (0.1 mL, 30%) and sample solution (0.1mL). After the mixture was stirred for 3min, the peroxide value was determined by reading the absorbance at 500 nm and the inhibition percentage of linoleic acid peroxidation was calculated as:

% inhibition = [1– (Test sample absorbance / Control absorbance)] × 100

All tests were run in duplicate and analysis of all sample was done in triplicate. α- tocopherol was used as the reference.

Reducing Power

This test was determined according to method used by Srinivas *et al*. [11]. Different concentrations of *S.nigrum* extract (100-400 µg) in 1mL of ddH_2O were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5mL, 1%). The mixture was incubated at 50°C for 20min. A portion (2.5mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000Xg for 10min. The upper layer of the solution (2.5mL) was mixed with ddH_2O (2.5mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

Statistical analysis

The data were expressed as mean values ± SEM. and all the data tested with analysis of variance followed by Dunnett's t-test. P-values < 0.05, 0.01 were considered to be statistically significant.

Results and Discussion

Anti-inflammatory study:

The results of this study for carrageenan and xylene tests are presented in Table 1 and 2, respectively. The extract at the doses of 200 and 400 mg/kg showed significant results ($P < 0.05$ and $P < 0.01$), respectively as compared to Diclofenac sodium (10mg/kg) and caused a significant inhibition in paw and ear edema volumes, respectively. Carrageenan induced inflammation in model to detect oral action of anti-inflammatory agents [12]. The development of oedema in the paw of the mice after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances [13]. The early phase (0.5-1 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandin in the damaged tissue surroundings. The late phase is sustained prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [14 ; 15]. The significant ameliorative activity of the ethanol extract and standard drug observed in the present study may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin. The carrageenan assay is a good method for the comparative bioassay of anti-inflammatory. The xylene-induced ear edema method [9], has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation.

Table 1 : Effect of the ethanol extract of *S. nigrum* on carrageenan induced paw edema in mice.

Groups	Dose mg/kg	Oedema diameter (cm)					Percentage of inhibition			
		0h	0.5h	1h	1.5h	2h	0.5h	1h	1.5h	2h
Group I	Control	0.82±0.01	0.88±0.03	0.9±0.03	0.96±0.02	1.01±0.01	—	—	—	—
Group II	Standard	0.64±0.02	0.62±0.04	0.59±0.07	0.57±0.02**	0.54±0.03**	46.54	40.63	34.45	29.46
Group III	100	0.80±0.08	0.76±0.01	0.74±0.06	0.71±0.04	0.70±0.03*	30.7	26.05	17.78	13.64
Group IV	200	0.75±0.07	0.70±0.03	0.69±0.05	0.66±0.01*	0.62±0.04*	38.62	31.25	23.34	20.46
Group V	400	0.68±0.03	0.65±0.02	0.64±0.02	0.61±0.05**	0.59±0.02**	41.59	36.46	28.89	26.32

N=6, values are mean ±SEM, *P<0.05, ** P<0.01, dunnet test as compared to control.

Table 2: Effect of the ethanol extract of *S. nigrum* on xylene- induced ear swelling in mice.

Groups	Dose mg/kg	Ear swelling (mg)	Percentage of protection
Group I	Control	6.7±0.51	—
Group II	Standard	3.6±0.62**	46.26
Group III	100	5.4±0.45	19.40
Group IV	200	4.8±0.52*	28.35
Group V	400	4.0±0.6**	40.29

N=6, values are mean ±SEM, *P<0.05, ** P<0.01, dunnet test as compared to control.

Antinociceptive study:

The results of this study for acetic acid induced writhing and hot plate tests are presented in Table 3 and 4, respectively. The ethanol extract at the doses of 100, 200 and 400 mg/kg caused an inhibition on the writhing response induced by acetic acid. The maximal inhibition of the nociceptive response was achieved at a dose of 400 mg/kg ($P < 0.01$). The oral dose of fruit extract at 400mg/kg ($P < 0.01$) elicited a significant analgesic activity as evidenced by increase in latency time on comparison with negative control at the end of 0.5, 1, 1.5 and 2 h. The increase in latency time was found in a dose dependent manner. Acetic acid causes an increase in the peritoneal fluid level of prostaglandins (PGE_2 & PGF_{2a}) as well as lipooxygenase products, involving in part peritoneal receptors and inflammatory pain by inducing

capillary [16 ; 17]. Collier *et al.*[18] postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate the nociceptive neurous. The important transmission pathways for inflammatory pain are that comprising peripheral polymodal nociceptors sensitive to protons, such as acid sensitive ion channels and to algogen substances, such as bradykinin and cytokines. Although the writhing test has poor specificity (e.g., anticholinergic, tricyclic antidepressants and antihistaminic and other agents show activity in this test), it is a very sensitive method of screening the antinociceptive of compounds [19 ; 20]. The hot-plate test is commonly used to assess narcotic analgesia. Although the central and peripheral analgesics respond by inhibiting the number of contractions provoked by chemical pain stimuli, only the central analgesics increase the time of response in the hot plate test [21]. These observations tend to suggest that the ethanol extract of *S. nigrum* may possess centrally- and peripherally-mediated antinociceptive properties. The peripheral antinociceptive effect of the extract may be mediated via inhibition of cyclooxygenases and/or lipooxygenases (and other inflammatory mediators), which its central antinociceptive action may be due its possible action as partial agonist of adrenergic, serotoninergic, cholinergic and dopaminergic receptors [22 ; 23].

Table 3: Effect of the ethanol extract of *S. nigrum* on latency to hotplate test.

Groups	Dose mg/kg	Mean latency (s) before and after drug administration					Percentage of inhibition			
		0h	0.5h	1h	1.5h	2h	0.5 h	1h	1.5 h	2h
Group I	Control	1.78±0.17	1.56±0.26	1.45±0.19	1.26±0.22	1.07±0.21	—	—	—	—
Group II	Standard	4.12±0.33**	5.49±0.1**	4.63±0.64**	3.68±0.62**	1.76±0.08	56.79	71.58	68.68	65.76
Group III	100	2.32±0.62	2.61±0.38	1.78±0.72	1.43±0.25	1.21±0.07	23.27	40.22	18.53	11.88
GroupIV	200	2.45±0.51	1.97±0.46	2.27±0.54	1.86±0.81	1.32±0.20	27.34	20.81	36.12	32.25
Group V	400	2.91±0.47	3.17±0.43**	4.35±0.32**	2.24±0.73	1.97±0.24	38.83	50.78	66.66	43.75

N=6, values are mean ±SEM, *P<0.05, ** P<0.01, dunnet test as compared to control.

Table 4 : Effect of ethanol extract of *S. nigrum* on acetic acid induced writhing in mice.

Groups	Dose mg/kg	No. of writhing	Percentage of protection
Group I	Control	36.2±0.44	—
Group II	Standard	6.4±0.39**	82.32
Group III	100	26.7±0.33*	26.24
Group IV	200	19.5±1.2**	46.13
Group V	400	15.8±1.02**	56.35

N=6, values are mean ±SEM, *P<0.05, ** P<0.01, dunnet test as compared to control.

Antioxidant activity:

The results of this study for antioxidant and reducing power tests are presented in Figure 1 and 2, respectively. The results revealed that the inhibition of peroxidation was progressively increased by raising the extract concentration and reached a plateau (about 83 %inhibition) when the concentration exceeded 400µg/mL ($P < 0.01$). The material extracts had overall good antioxidant activity. A direct correlation between antioxidant capacity and reducing power of the extract has been reported. The reducing properties are generally associated with the presence of reductions, which have been show to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [24]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals [25].

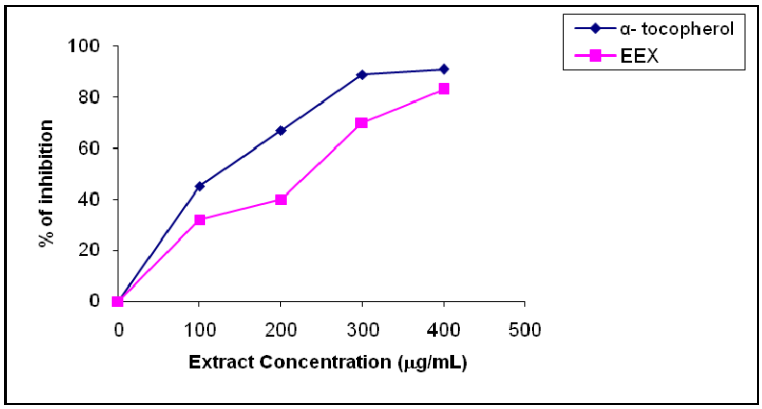

Figure 1. Antioxidant activity of the ethanol extract, in comparison with α-tocopherol.

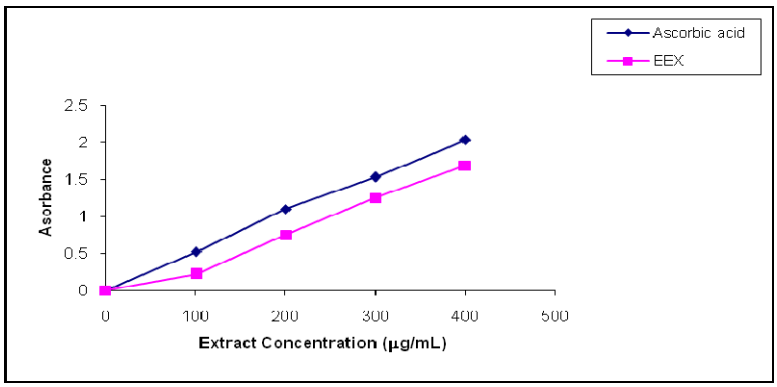

Figure 2. Reducing power of the ethanol extract, in comparison with ascorbic acid.

Conclusion

Based on the results of the present study, we conclude that the plant extract possesses strong anti-inflammatory, analgesic and antioxidant potential. Further studies are necessary to elucidate the mechanisms behind its effects and the results suggest that the inhibition of lipid peroxidation is likely to be a component of its anti-inflammatory activity.

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