IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2023 Vol.:26, Issue:4

Evaluation of Anti-Arthritic, Anti-Infllamatotry and Analgegesic Activities of *Solanum nigrum* Extract

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Saeeda Baloch[1], Shafi Muhammad*[1], Abdul Jabbar[1], Nagina Soomer Khan[3], Muhammad Younis[2], Abdul Bari[2], Taufiq Ahmed[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan. [2]Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan. [3]Department of Eastern Medicine, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan.

Submitted: 22 February 2023
Accepted: 28 February 2023
Published: 30 March 2023

Keywords: Anti-arthritic activity, methanolic extract, *Solanum nigrum*

ABSTRACT

Arthritis, disease of joint mainly caused by constant swelling about the joint, causes pain, thickness and increase in synovial cell membrane, extra growth in joints and morphological changes. Natural sources are the only source of new drugs, Hence the current investigation was intended to evaluate the phytochemical tests, acute toxicity, anti-arthritic, anti-inflammatory and analgesic activities of *Solanum nigrum* methanol extract. In phytochemical tests results were positive for the presence of saponins, flavonoids, alkaloids and steroids. In acute toxicity test oral administration of the *S. nigrum* extract from 100 to 2g/kg showed 10% mortality. In anti-arthritic activity, *S. nigrum* methanol extract significantly ($p<0.05$) reduced the edema of rat joint paw as compared with control and arthritic control. Results revealed that *S. nigrum* extract significantly ($p<0.05$) suppressed the rat paw oedema from 1 to 4 h after carrageenan injection. In analgesic activities *S. nigrum* extract, significantly ($p<0.05$) reduced the number writhes in acetic acid induced writhing test and significantly ($p<0.05$) increased the withdrawal time of tail in tail immersion test. It is concluded that *S. nigrum* extract showed significant anti-arthritic, anti-inflammatory and analgesic activities.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

According to WHO data, rheumatoid arthritis affects 0.3-15 percent of the global population and it is three times extra recurrent in women than in men. Rheumatoid arthritis is an inflammatory disease that causes persistent inflammation in the bones and joints of the body. The symptoms of RA include swelling, discomfort, bone and cartilage deterioration, and eventually lifelong disability. The exact reason is unclear; however some theories suggest that it is triggered by genetic susceptibility and that environmental elements such as viruses are also at blame[1].

Plant-based medicines have been used by humans since the ancient time; the history of using plants for therapeutic purposes is as old as humanity itself [2]. Herbal medications are created using therapeutic knowledge passed down through generations of early treatment system healers[3]. Because of their lower toxicity and ease of availability, many scientists are now focusing on natural products[4]. Nature has blessed mankind with a vast wealth of medicinal plants that are widely distributed throughout the universe and used as therapeutic agents for the treatment and prevention of a wide range of ailments[5, 6].

Solanum nigrum L. belonging to solanaceae family. *S. nigrum* L. is also a medicinal herb utilized skin problems. Previous studies shows that *S. nigrum* L. contains antibacterial, antioxidant and anti-inflammatory effects[7,8,9,10]. Flavonoids and polyphenols have been reported from *S. nigrum* [11,12]. Hepatoprotective and antitumor effects have also been reported[13].

In Balochistan, *S. nigrum* is utilized in traditional medicine. Present study was aimed to evaluate the phytopharmacological activities of *S. nigrum* crude methanolic extract.

MATERIAL AND METHODS

Plant Material

Plant material was collected from Mastung district of Balochistan, and then kept for drying under shade for 15 days. Dried plant material was soaked in methanol for 15 days and then the solvent was removed by using rotary evaporator.

Animals

Experimentations was done on female and male Sprague-Dawley rats, about 200-250 g weight. Animals remained in controlled temp room (21° ± 2°C) and humidity (55 ± 5%)was also maintained. The animals remained in particular experimental conditions in the animal house facility FOPHS.

Preliminary Phytochemical tests

Test for the presence of Alkaloid, tannins, resins, glycosides, flavonoids and terpenoids were carried out according to standard protocol [14].

Acute oral toxicity

The method was completed in accordance with OECD 423 principles (OECD/OCDE, 2002). In order to investigate acute toxicity, we administered the *S. nigrum* extract from 100mg/kg to 2000mg/kg by gastric gavages. To account for the possibility of animal death, the animals were monitored for a period of 14 days [15].

Anti-Arthritic activity

Freund adjuvant (CFA) induced arthritis in rats .

Rats remained distributed in Five (05) groups. 1st group was treated with 03ml/kg of distilled water. 2nd group remained arthritic control. 3rd and 4th group remained treated with *S. nigrum* crude methanolic extract 250 mg and 500mg/kg respectively. 4th grouped remained treated with Aspirin,100 mg/kg, orally. For Arthritis induction CFA (0.05 ml) was injected beneath skin of rats left foot pad. Treatments were started 1 day before of CFA injection and regularly sustained for fourteen (14) days. By using digital vernier caliper, the edema of injected paw was measured at 1st , 5th , 10th and 15th days, afterwards CFA injection [16].

Anti-inflammatory activity

Carrageenan-induced paw edema in rats

Wistar (male) rats (about 210–230 g) distributed in four (04) groups containing 06 rats each. Control group was treated with distilled water 3ml/kg. *S. nigrum* 250 and 500 mg/kg (2nd and 3rd group) and diclofenac sodium 50 mg/kg (standard drug) in 0.6% CMC were orally administered 1 hour earlier carrageenan injection. 1% carrageenan (0.1ml) was

subcutaneously injected in the plantar side of right hind paw of rat. With the help of digital vernier caliper, thickness of paw was measured, firstly (0 hour) and then at 1st , 2nd , 3rd , 4th , 5th and 6th hour afterwards of carrageenan injection [17].

Analgesic activity

Writhing test with acetic acid

An animal model was used to predict the pain-relieving properties of *S. nigrum* crude methanolic extract. Group I (control) and Groups II & III (*S. nigrum* extract 250 and 500mg/kg treatment groups) and Group IV (standard drug treated group.) Afterward thirty (30) min test control vehicle , extract drug and standard drug, 0.7% acetic acid was injected intraperitoneally in right hind paw of mice. Number of writhes were counted for 30 minutes [18].

Tail immersion test

Four sets of rats remained used (5 rats in each group). Distil water 1ml/kg was administered to control group. Group II and III (treated with *S. nigrum* extracts at 250 and 500mgkg^{-1}, separately). Diclofenac sodium (50mgkg^{-1}) was given to group IV. We monitored each rat's "pain response time" (PRT) after administering each treatment by immersing their tails in a warm water bath (temperature maintained at 55±02 ^{0}C) for 15 seconds, after which they had to get their tails out of the water or flick them[19].

Statistical Analysis

Data were evaluated using ANOVA (ANOVA) and Dunnett's multiple comparison test using SPSS software (version 22). P value < 0.05 is considered significant and p < 0.01, considered highly significant [20].

RESULTS

Phytochemicals

Methanolic extracts of *Solanum nigrum* were analyzed for presence of phytochemicals and the results presented in table-1. Data indicates that *S. nigrum* extract were found positive for presence of four compounds i.e. saponins, flavonoids, alkaloids and steroid.

Acute toxicity of *S. nigrum* extract

Results on the acute toxicity of *S. nigrum* extract is mentioned in table-2. Data indicates that *S. nigrum* extract at dose of 2g/kg showed 10% mortality. The *S. nigrum* extract at dose of 100mg/kg, 150mg/kg, 200mg/kg, 250mg/kg, 500mg/kg and 1g/kg did not showed any sign of toxicity.

Anti-Arthritic activity

Freund adjuvant (CFA) induced arthritis in rats.

In anti-arthritic activity, *S. nigrum* methanol extract significantly ($p<0.05$) reduced the edema of rat joint paw as compared with control and arthritic control.

Anti-inflammatory activity

Carrageenan-induced paw edema in rats

Results on the rat paw odema is mentioned in table-4. Data indicates that *S. nigrum* extract significantly suppressed the rat paw oedema from 1 to 4 h after carrageenan injection.

Analgesic activities

Acetic acid induced writhing activity

Results on the acetic acid induced writhing test is presented in table-5. Data indicates that mean number of writhes for control group were 77.2±0.58. The mean number of writhes for *S. nigrum* extract at dose of 250 and 500 mg were observed as 67.2±1.11 and 59.18±0.59, respectively. For standard drug (Diclofenac sodium), mean number of writhes was recorded as 40.51±0.48. The analysis of data indicates significant ($p<0.05$) decrease was observed in number of writhes between control and drug treatment groups.

Tail immersion test

Results on the tail flick assay is presented in table-6. Data indicates that mean time (seconds) for control group were 6.18±0.05. The mean time (seconds) for *S. nigrum* extract at dose of 250 and 500 mg were observed as 8.2±0.12 and 7.56±0.06, respectively. For standard drug (Diclofenac sodium), mean time (seconds) was 9.58±0.13. The analysis of data indicates significant ($p<0.05$) increase in mean time of tail flick as compared with control group.

DISCUSSION

Rheumatoid Arthritis, which causes swelling in the joints and systemic changes, is a more common illness with substantial systemic clinical consequences and a high death rate in patients when compared to healthy persons. Synovium swelling associated with synovial-cells proliferation, thought to be the primary cause of cartilage affection and deterioration in rheumatoid arthritis [21]. Bone degradation affects 80% of patients and happens quickly when there is high and sustained inflammation [22]. Osteoclast differentiation and periosteal intrusion found at the surface next to articular cartilage are caused by cytokines secreted in the synovium, primarily IL-1. TNF- and interleukin 1 and 6 also enhance the differentiation and activation of osteoclasts. In addition, suppression of TNF-, interleukin-1, and RANKL in therapeutic therapy may postpone bone degradation[23].

The current investigation shows that methanolic extracts of *S. nigrum* produced toxicity at a level of 2g/kg resulted in 10% mortality. The polyarthritis model generated by CFA in rats is frequently utilized in evaluation drugs for anti-arthritis effects. This method is commonly utilized for inflammation diseases and is effective model for pain, owing to its strong resemblance to actual rheumatoid disorders [24]. Extracts of *S. nigrum* exhibited antiarthritic potential in inflammatory parameters, results were in accordance with previous studies [25]. Methanolic extracts of *S. nigrum* considerably reduced rat paw oedema from 1 to 4 hours after carrageenan injection, according to the findings the *S. nigrum* extract reduced inflammation in treated animals. The analgesic effect of *S. nigrum* extract in rats with adjuvant arthritis was also noticeable, as shown by a considerable reduction number of writhes as compared with control and standard drug. It was also found that *S. nigrum* substantially enhanced the response time of the animal on the tail flick assay. In phytochemical studies *S. nigrum* extract the results were positive for presence flavonoids, alkaloids and steroids, and these compounds are may be responsible for above mentioned activities.

CONCLUSION

It is concluded that *S. nigrum* methanolic extract produced significant anti-arthritic, anti-inflammatory and analgesic activities with low toxicity profile in acute toxicity test. Nevertheless, supplementary experiments needed for isolation of the chemical compounds accountable for pharmacological effects.

Table-1 Phytochemicals present in methanolic extract of *Solanum nigrum*

S.No.	Phytochemicals	Results
1.	Saponins	+
2.	Tannins	-
3.	Flavonoids	+
4.	Alkaloids	+
5.	Steroid	+
6.	Glycosides	-

-=negative; +=positive

Table-2 Acute toxicity of methanolic extracts of *Solanum nigrum*

S.No.	Dose	% mortality
1	*S. nigrum* 100 mg/kg	0
2	*S. nigrum* 150mg/kg	0
3	*S. nigrum* 200mg/kg	0
4	*S. nigrum* 250mg/kg	0
5	*S. nigrum* 500mg/kg	0
6	*S. nigrum* 1g/kg	0
7	*S. nigrum* 2g/kg	10

Table-3 Ant-arthritic activity (Freund adjuvant (CFA) induced arthritis in rats)

S.No.	Drug	Day 1	Day5	Day15	Day 20
1	Control	5.23±0.10	5.40±0.14	5.43±0.06	5.48±0.13
2	Arthritic Control	9.04+0.27	19.29±0.14	24.75±0.23	28.28±0.15
3	SN 250mg	7.29±0.03	14.60±0.19	21.73±0.09	24.66±0.07
4	SN 500mg	6.78±0.16	12.76±0.11	15.88±0.38	18.36±0.14
5	Standard drug (Aspirin 100mg/kg)	6.12±0.12	10.73±0.18	12.35±0.14	14.43±0.15

SN= *Solanum nigrum*

Table- 4 Rat paw odema

S.No.	Drug	0 h	1h	2h	3h	4h
1	Control	166.94±0.90	311.65±0.57	292.47±47	282.24±0.57	272.93±0.89
2	SN 250mg	194.55±1.39	308.21±0.70	273.57±0.88	256.02±0.52	243.91±0.31
3	SN 500mg	198.46±0.37	305.02±0.22	262.41±0.86	242.30±0.43	228.64±0.41
4	Standard drug (Diclofenac sodium)	192.42±0.73	290.72±0.70	248.63±0.26	234.42±0.26	220.47±0.56

SN= *Solanum nigrum*

Table- 5 Acetic acid induced writhing activity.

S.No.	Drug	Number of writhes
1	Control	77.2±0.58
2	SN 250mg	67.2±1.11
3	SN 500mg	59.18±0.59
4	Standard drug (Diclofenac sodium)	40.51±0.48

SN= *Solanum nigrum*

Table-6 Tail flick assay

S.No.	Drug	Number of activities
1	Control	6.18±0.05
2	SN 250mg	8.2±0.12
3	SN 500mg	7.56±0.06
4	Standard drug (Diclofenac sodium)	9.58±0.13

SN= *Solanum nigrum*

REFERENCES

1. Choudhary M, Kumar V, Malhotra H, Singh S. Medicinal plants with potential anti-arthritic activity. Journal of intercultural ethnopharmacology. 2015 Apr;4(2):147..
2. Tandon V, Gupta RK. Histomorphological changes induced by Vitex negundo in albino rats. Indian journal of pharmacology. 2004 May 1;36(3):176.
3. Vispute S, Khopade A. *Glycyrrhiza glabra* Linn.-"Klitaka": A review. Int J Pharm Bio Sci. 2011;2:42–51.

4. Badami S, Moorkoth S, Suresh B. *Caesalpinia sappan* a medicinal and dye yielding plant. Nat Prod Rad. 2004;3:75–82.
5. Kalaria P, Gheewala P, Chakraborty M, Kamath J. A phytopharmacological review of *Alstonia scholaris*: A panoramic herbal medicine. IJRAP. 2012;3:367–371.
6. Sharangi AB. Medicinal and therapeutic potentialities of tea (Camellia sinensisL.)-A review. Food Res Int. 2009;42:529–535.
7. Wang Y, Xiang L, Yi X, et al. Potential anti-inflammatory steroid saponins from the berries of *Solanum nigrum* L. (European Black Nightshade). J Agr Food Chem. 2017;65:4262–4272
8. Zhao Z, Jia Q, Wu MS. Degalactotigonin, a natural compound from *Solanum nigrum* L., inhibits growth and metastasis of osteosarcoma through GSK3β inactivation–mediated repression of the Hedgehog/Gli1 pathway. Clin Cancer Res. 2017;24:130–144.
9. Lafuente AG, Guillamón E, Villares A, et al. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res. 2009;58:537–552
10. Son YO, Kim J, Lim JC, et al. Ripe fruits of *Solanum nigrum* L. inhibits cell growth and induces apoptosis in MCF-7 cell. Food Chem Toxicol. 2003;41:1421–1428.
11. Viuda-Martos M, Ruiz-Navajas Y, FernándezLópez J, et al. Functional properties of honey, propolis, and royal jelly. J Food Sci. 2008;73:117–124.
12. Nasuti C, Gabbianelli R, Falcioni G, et al. Antioxidative and gastroprotective activities of anti-inflammatory formulations derived from chestnut honey in rats. Nutr Res. 2006;26:130–137.
13. Patel A., Biswas S, Shoja M H, Ramalingayya V & Nandakumar K Protective effects of aqueous extract of *Solanum nigrum* Linn. leaves in rat models of oral mucositis. *The Scientific World Journal*, *2014*.
14. Tiwari RK, Chanda S, Singh M, Agarwal S. Anti-inflammatory and anti-arthritic potential of standardized extract of Clerodendrum serratum (L.) Moon. Frontiers in Pharmacology. 2021 Apr 12;12:629607.
15. Younis M, Iqbal J, Muhammad S, Jan SU, Jabbar A, Qadir A, Arslan M, Shah PA, Khan Z. Evaluation of anti-inflammatory and analgesic effects of Hertia intermedia (Boiss.) Kuntze extract. Pakistan Journal of Pharmaceutical Sciences. 2022 Jul 1;35(4 (Special)):1281-6.
16. Uttra AM, Hasan UH. Anti-arthritic activity of aqueous-methanolic extract and various fractions of Berberis orthobotrys Bien ex Aitch. BMC complementary and alternative medicine. 2017 Dec;17(1):1-6.
17. Simon BI, Lidianys ML, Armida AG, Claudia LL, Daniela FA, Jose LR, Rene BR. Anti-inflammatory activity and modulate oxidative stress of Bucida buceras in lipopolysaccharide-stimulated RAW 264.7 macrophages and Carrageenan-induced acute paw edema in rats. Journal of Medicinal Plants Research. 2017 Mar 25;11(12):239-52.
18. Bukhari IA, Gilani AH, Meo SA, Saeed A. Analgesic, anti-inflammatory and anti-platelet activities of Buddleja crispa. BMC complementary and alternative medicine. 2016 Dec;16(1):1-7.
19. Kumar JP, Shankar NB. Analgesic activity of Mollugo pentaphylla Linn. by tail immersion method. Asian J. Pharm. Clin. Res. 2009;2(1):61-3.
20. Khan IA, Hussain M, Hussain N, Alqahtani AM, Alqahtani T. Cardioprotective Effect of Rumex vesicarius Linn. Leaf Extract against Catecholamine-Induced Cardiotoxicity. Molecules. 2022 May 24;27(11):3383.
21. Rhee DK, Marcelino J, Baker M, et al. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. J Clin Invest. 2005;115:622–31.
22. Visser H, Le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis & Rheumatism. 2002 Feb;46(2):357-65.
23. Hess A, Axmann R, Rech J, Finzel S, Heindl C, Kreitz S, Sergeeva M, Saake M, Garcia M, Kollias G, Straub RH, Sporns O, Doerfler A, Brune K, Schett G. Blockade of TNF-α rapidly inhibits pain responses in the central nervous system. Proc Natl Acad Sci U S A. 2011;108(9):3731–6
24. Singh S, Majumdar DK. Effect of fixed oil of Ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals. Int J Pharmacogn. 1996;34:218–2
25. Mbiantcha M, Almas J, Shabana SU, Nida D, Aisha F. Anti-arthritic property of crude extracts of Piptadeniastrum africanum (Mimosaceae) in complete Freund's adjuvant-induced arthritis in rats. BMC complementary and alternative medicine. 2017 Dec;17(1):1-6.

Image *Author -1*	Saeeda Baloch Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
Image *Author -2*	Shafi Mhammad Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
Image *Author -3*	Abdul Jabbar Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
Image *Author -4*	Nagina Soomer Khan Department of Eastern Medicine, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
Image *Author -5*	Muhammad Younis Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
Image *Author -6*	Abdul Bari Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
Image *Author -7*	Taufiq Ahmad Department of Eastern Medicine, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan