SJIF Impact Factor: 6.711

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 8.
Issue: 4
Page N. 143-148
Year: 2024

Research Article www.wjahr.com

INVIVO PHARMACOLOGICAL SCREENING OF HERBAL EXTRACTS AGAINST NEUROPATHIC PAIN

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Article Received date: 20 February 2024 **Article Revised date:** 11 March 2024 **Article Accepted date:** 01 April 2024

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ABSTRACT

Neuropathic Pain (NP) is defined as "Pain that is brought on by a primary nervous system lesion or dysfunction." It is majorly observed as co-morbidity with diabetes, rheumatoid arthritis and others. A few drugs are used in treatment with side effects. Recent investigations are focusing light on NP. *Solanum melongena* and *Solanum lycopersicum* are popular medicinal herbs employed for treating various diseases. The present research focuses on screening of leaves of *Solanum melongena* and *Solanum lycopersicum* for neuropathic pain. The extraction is performed using continuous hot percolation with ethanol as menstrum. The extracts are subjected for phytochemical screening in order to evaluate the type of phytoconstituent present in it. Screening for neuropathic pain property is performed using Formalin induced paw flinching behaviour and AITC induced paw oedema models. Formalin and AITC acts as inducers for neuropathic pain by stimulating TRP channels in nerves. The inflammatory volume observed in group of animals treated with *Solanum melongena* leaf extract at dose of 400mg/kg showed maximum activity in both the models indicating a decline in oedema and flinching behaviour compared with other extracts. A mild increase in response is observed when compared with standard values. The *Solanum melongena* leaf extract at dose of 400mg/kg showed good Neuropathic Pain property.

1. INTRODUCTION

1.1 Introduction to pain

Pain is characterized as an unpleasant emotional and sensory experience that is linked to or explained in terms of tissue damage, whether it is actual or potential. According to estimates, 20% of adults worldwide experience pain and 10% receive a new diagnosis of chronic pain each year. Pain is a major global issue.[1]

Pain is a sensation in one or more body parts but it is also usually unpleasant, making it an emotional experience as well. sensations that mimic pain, such as prickling, dysesthesia or unpleasant atypical feelings can also be pain, although they don't always have to be and they might not have the same sensory characteristics.[2]

According to the International Association for the Study of Pain (IASP), Neuropathic Pain (NP) is defined as "Pain that is brought on by a primary nervous system lesion or dysfunction."

The following traits would characterize NP and set it apart from other forms of pain are.

1. Sensual and pain symptoms that last after the healing phase.
2. Neurological sensory indicators that appear as both positive and negative sensory events are present, varying in intensity.
3. Other neurological symptoms, such as motor ones that present as both positive and negative motor phenomena or autonomic signals, may be present to varying degrees.[3]

1.2 Introduction to plants

Plants and plant products are recognized to have important therapeutic qualities. Historically, people have employed medicinal plants as a traditional means of treating a variety of illnesses. Strong and efficient analgesic medications are nonetheless required, notwithstanding the advancements made in treatment development. In this sense, it has been amply demonstrated that a variety of compounds produced from plants are important in the process of creating novel approaches to treating pain-related issues.[4]

Outside traditional medicine, the use of herbal medicine is well-established. Clinical research is progressing and analysis and quality control are improving, leading to the use of herbal medicines for both cure and preventative purposes. Because herbal medications are far less expensive and have fewer side effects than conventional pharmaceuticals, using such alternative medicine would aid the impoverished in recovering from their illnesses.[5]

The efficacy of plant-based medications used in traditional medicine has drawn a lot of attention due to the possible side effects and inefficiency of chemical and synthetic drugs. Herbal medicines are considered safe, have good efficacy, are accepted in culture, and have fewer side effects than synthetic drugs.[6]

1.3 Introduction to *Solanum lycopersicum*

Solanum lycopersicum(tomato), a member of the Solanaceae has served as an important model system for studying the genetics and molecular basis of resistance mechanisms in plants. There are around 3000 species in the Solanaceae family, popularly known as the nightshade family, with a wide range of morphological, habitat and behavioral variations.[7]

Figure 1: Figure showing the Fruits and Leaves of *Solanum lycopersicum* plant.

1.3.1 Scientific classification

Kingdom : Plantae.
Phylum : Angiosperms
Order : Solanales
Family : Solanaceae
Genus : *Solanum*
Species : *lycopersicum*.[8]

Tomato is produced in temperate, subtropical and tropical areas around the world and it is the second horticultural crop produced in terms of yield in the world. Since 2016 United States, China, India and Turkey are the countries with the largest production area.[9] On an area of around 3,170,000 hectares, the world produces roughly 89.8 million metric tons of tomatoes, with Nigeria being the continent's second-largest producer.[10]

Solanum lycopersicum, or the tomato, is one of the most significant vegetable plants in the world. It is believed to have originated in western South America where the domestication took place in Central America. Tomatoes are important food crops, so they have been developed to increase fruit quality, productivity and tolerance to biotic and abiotic stressors. Tomatoes are frequently used as study materials in addition to being used as food.[11]

1.4 Introduction to *solanum melongena*

The crop *Solanum melongena* is a member of the Solanaceae family. It is frequently referred as eggplant. This shrub has many branches and grows to a height of two meters. Its taproot is extensive and penetrates deeply into the ground. Star-shaped hairs and occasionally prickles cover the stems and leaves profusely. It features a meaty, berry fruit with many seeds that is typically smooth and shiny around the world i.e. eggplant is grown.

Fig. 2: Figure showing the Leaves and Fruits of *solanum melongena.*

The herbaceous annual grows in an upright or semi-spreading manner. Growing to a height of 60-120 cm, it becomes a bushy shrub with big leaves. In most cultivars, the underside is caked with dense hair that resembles wool and the leaves have an opposing, huge, single-lobed pattern. In the middle of the leaf, there may or may not be spines.[12]

1.4.1 Scientific classification

Kingdom : Plantae
Subkingdom : Viridaeplantae
Infrakingdom : Streptophyta
Division : Tracheophyta
Subdivision : Spermatophytina
Infradivision : Angiospermae
Class : Magnoliopsida
Subclass : Asteridae
Order : Solanales
Family : Solanaceae
Genus : *Solanum* L.
Species : *Solanum melongena* Linn.

Eggplant in addition to its beneficial dietary numerous researchers have validated that the fruit's skin contains nasunin, anthocyanin phytonutrient, which is a strong antioxidant. It has been discovered that eggplant lowers LDL because it contains phenolic chemicals. Eggplant

leaf extract is more effective against skin fungi that cause disease in humans.

Alkaloids like nicotine, flavonoids, saponins and resins are found in eggplants. Specifically, nicotine, an alkaloid present in all species of *S. melongena*, conferred the analgesic function. There are hydrocaffein, protocatechuric and chlorogenic acids in leaves. Tropane, quinazolizidine, pyrrolidine, glycoalkaloids and steroid alkaloids are a few of the alkaloids that are found. Because it contains tannins, flavonoids and alkaloids, the dry residue of *S. melongena* leaves has a dose-dependent analgesic effect.[13]

2. MATERIALS AND METHODS

2.1 Plant material

The plant components, included are leaves of *Solanum melongena* and *Solanum lycopersicum*, that were procured from nearby farms and are authenticated from Acharya Nagarjuna University located in Andhra Pradesh, India.

2.2 Plant extraction

The leaves of *Solanum lycopersicum* and *Solanum melongena* were allowed to shade dry. The leaves were ground into a fine powder after drying. To fill the porous cellulose thimble, there should be enough plant material. The Soxhlet extractor's thimble, holds the crushed plant material. Glass wool is used to lag the side arm. The ethanol (Solvent) passes through the apparatus to the condenser after being heated with the help of the heating mantle. The evaporated solvent from round bottom flask and the condensate pours it into the reservoir holding the thimble. The cycle restarts when the solvent level hits the siphon and flows back into the flask. A total of sixteen hours should be spent on this process. When it is not allowed to run the equipment overnight, it can be turned on and off and the time can be divided across several days. After the procedure is complete, the ethanol should be removed from the plant material using a rotary evaporator, leaving a tiny amount (about 2 to 3 ml) in the glass bottom flask.[14,15]

Fig. 3: Figure showing soxhlet extraction of herbs.

2.3 Preliminary phytochemical analysis

The sample was processed in compliance with the guidelines. The two extracts are separately mixed with a minor quantity of water and subjected to phytochemical screening to estimate the presence of alkaloids, saponins, tannins, flavonoids and proteins.

2.4 Animals

Conventional animal house circumstances, the animals were housed in plastic cages with a temperature range of 28 to 31°C, a photoperiod of about 12 hours of natural light each day and a relative humidity of between 50% and 55%. Water and pelleted food were freely available to all of the rats. Globally recognized guidelines for the use and care of animals were followed when conducting studies on animals. Every attempt was made to lessen the amount of pain experienced by animals and to use fewer animals overall.[16,17,18]

2.5 Formalin induced paw flinching or licking behaviour

Albino wistar rats were randomly divided into 6 groups and were orally administered with standard drug, test drug extracts and vehicle as follows: group 1 (n=6) serves as control and treated with vehicle; group 2 (n=6) serves as standard and treated with Gabapentin (10mg/kg); group 3 (n= 6) treated with 200 mg/kg of *Solanum melongena* leaf extract; group 4 (n= 6) treated with 400 mg/kg *Solanum melongena* leaf extract, group 5 (n=6) treated with 200mg/kg of *solanum lycopersicum* extract and group 6 (n=6) treated with 400mg/kg of *solanum lycopersicum* extract. Half an hour after administration, each rat was subcutaneously injected with 0.05µl 2.5% formalin solution into the sub plantar surface of the left hind paw. Rats were then observed for a duration of 60 min and the number of flinches in treated hind paw and the duration of licking time was recorded.[19]

2.6 AITC induced paw oedema

After an adaptation period (15 min), 20µl of 0.1% (w/w) AITC solution was injected into the dorsal surface of the right hind paw of each rat. Group 1 (n=6) serves as control and treated with vehicle; group 2 (n=6) serves as standard and treated with Gabapentin (10mg/kg) ; group 3 (n= 6) treated with 200 mg/kg of *Solanum melongena* leaf extract; group 4 (n= 6) treated with 400 mg/kg *Solanum melongena* leaf extract, group 5 (n=6) treated with 200mg/kg of *solanum lycopersicum* extract and group 6 (n=6) treated with 400mg/kg of *solanum lycopersicum* extract. The standard and test compounds were administered 15 min prior before AITC injection. In this test, the animals were observed individually for 20 min following AITC injection. Inflammatory pain-related behaviour i.e. oedema, was measured using a plethysmometer and the rise in volume of mercury was noted.[20]

3. RESULTS

3.1 The results of phytochemical screening of *solanum melongena*

Table 1: Table showing phytochemical screening result of ethanolic extract of *Solanum melongena.*

S. No.	Test	Result	Observation
1	Mayers test	++	Presence of alkaloids
2	Benedict's test	+	Presence of carbohydrates
3	Biuret test	−	Absence of proteins
4	Liebermann Burchard test	++	Presence of steroids
5	Ferric chloride test	++	Presence of phenols
6	Sodium hydroxide test	++	Presence of flavonoids
7	Salkowski test	++	Presence of terpenoids
8	Keller Killian test	+	Presence of glycosides

(+)- Presence of compound (−)- Absence of compound

3.2 The results of phytochemical screening of *Solanum lycopersicum*

Table 2: Table showing phytochemical screening result of ethanolic extract of *Solanum lycopersicum.*

S. No.	Test	Result	Observation
1	Mayers test	+	Presence of alkaloids
2	Benedict's test	+++	Presence of carbohydrates
3	Biuret test	++	Presence of proteins
4	Liebermann Burchard test	+	Presence of steroids
5	Ferric chloride test	+++	Presence of phenols
6	Sodium hydroxide test	++	Presence of flavonoids
7	Salkowski test	+	Presence of terpenoids
8	Keller Killian test	+	Presence of glycosides

(+)- Presence of compound (−)- Absence of compound

3.3 Results of formalin induced paw flinching behaviour

The table listed below indicates the time taken for the animals in group to show paw flinching behaviour.

Table 3: Table showing number of flinches observed for formalin induced paw flinching behaviour model.

Groups	Drug treated	Total number of flinches in 1 hour
Group - 1	Control	135.33 ± 2.16 (ns)
Group - 2	Standard	35.5 ± 1.878
Group - 3	200mg/kg of S*olanum melongena* leaf extract	73.33 ± 2.34*
Group - 4	400mg/kg of S*olanum melongena* leaf extract	37 ± 2.36*
Group - 5	200 mg/kg of S*olanum lycopersicum* leaf extract	87.5 ± 1.87*
Group - 6	400 mg/kg of S*olanum lycopersicum* of leaf extract	42 ± 2.36*

P < 0.001 – Highly significant, P < 0.01 – Significant and P > 0.05 – Non significant

3.4 Results of AITC induced paw oedema

The table listed below indicates the volume of mercury rise in plethysmograph with respect to time.

Table 4: Table showing rise in volume of mercury in plethysmograph on AITC induced paw oedema model.

Groups	Drug treated	Volume of Mercury rise in Plethysmograph						
		0hr	0.5hr	1hr	2hr	3hr	4hr	5hr
Group - 1	Control	0	0.2	0.25	0.45	0.6	0.7	0.8
Group - 2	Standard	0	0.05	0.1	0.05	0.05	0	0
Group - 3	200mg/kg of S*olanum melongena* leaf extract	0	0.15	0.2	0.3	0.45	0.6	0.4
Group - 4	400mg/kg of S*olanum melongena* leaf extract	0	0.1	0.15	0.05	0.05	0.05	0.05
Group - 5	200 mg/kg of S*olanum lycopersicum* leaf extract	0	0.2	0.25	0.35	0.5	0.65	0.5
Group - 6	400 mg/kg of S*olanum lycopersicum* of leaf extract	0	0.15	0.2	0.15	0.1	0.1	0.1

4. DISCUSSION

Solanum melongena and *Solanum lycopersicum* are popular medicinal herbs employed for treating various diseases. The present research focuses on screening of leaves of *Solanum melongena* and *Solanum lycopersicum* for nociceptive pain.

Fresh leaves are collected from crops and fields and shade dried. After drying the leaves are grinded into fine powder for extraction. Extractions are done with ethanol for 3 constitutive days, followed by vacuum filtration. The extracts are made solvent free by evaporation to separate extracts. Ethanolic extracts of *Solanum melongena* and *Solanum lycopersicum* were about 4.0gms and 3.5gm.

The extracts are subjected for phytochemical screening to identify the presence of compounds in it. The *Solanum melongena* extract contains carbohydrates, amino acids, alkaloids, phenols, steroids, alcohol and absence of proteins. The *Solanum lycopersicum* extract contains carbohydrates, amino acids, alkaloids, phenols, steroids, alcohol, proteins and absence of resins.

Screening for neuropathic pain property is performed using Formalin induced paw flinching behaviour and AITC induced paw oedema models. Formalin and AITC acts as inducers for neuropathic pain by stimulating TRP channels in nerves. The time taken by animals to show paw flinching behaviour and volume of mercury rise in plethysmograph were observed.

Animals are divided into 6 groups with each group containing 6 animals. Control group received saline and standard group received Gabapentin along with inducer 5% formalin solution. Control group without drug and only inducer showed paw flinching response for about 135.33 ± 2.16sec for more duration as there is no drug given where as for standard group receiving Gabapentin it took **35.5 ± 1.87 sec.** Test groups are treated with 200 and 400mg/kg of both ethanolic extracts of *Solanum melongena* and *Solanum lycopersicum*. The groups receiving ethanolic extract of *Solanum melongena* 400mg/kg dose show good response compared with 200mg/kg and standard. Among the higher dose test groups, the group receiving ethanolic extract of *Solanum melongena* at 400mg/kg dose showed maximum paw flinching behaviour (37 ± 2.36).

Animals are divided into 6 groups each containing 6 animals each. Control group received saline and standard group received Gabapentin along with inducer AITC. Control group without drug and only inducer the volumes of mercury rise in plethysmograph are 0, 0.2, 0.25, 0.45, 0.6, 0.7 and 0.8ml at 0, 0.5, 1, 2, 3, 4 and 5hrs, where as for standard group receiving Gabapentin it took 0, 0.05, 0.1, 0.05, 0.05, 0 and 0ml at 0, 0.5, 1, 2, 3, 4 and 5hrs. Test groups are treated with 200 and 400mg/kg of both ethanolic extracts of *Solanum melongena* and *Solanum lycopersicum*. Among the higher dose test groups, the group receiving ethanolic extract of *Solanum melongena* at 400mg/kg dose showed the volumes rise in plethysmograph are 0, 0.1, 0.15, 0.05, 0.05 and 0.05ml at at 0, 0.5, 1, 2, 3, 4 and 5hrs.

5. SUMMARY AND CONCLUSION

Solanum melongena and *Solanum lycopersicum* are medicinal herbs and used in treating various diseases. The present research focuses on investigating plants to treat nociceptive pain. Leaves of two species from Solanaceae are selected for screening of nociceptive pain.

Fresh leaves of *Solanum melongena* and *Solanum lycopersicum* are collected, dried, grinded and extracted with ethanol by continuous hot percolation. The extracts are made solvent free and subjected for phytochemical screening to evaluate the presence of carbohydrates, alcohols, phenols, proteins and steroids.

Formalin induced paw flinching behaviour and AITC induced paw oedema models were performed to find out the nociceptive property. Animals are divided into six groups treated with saline, Gabapentin, ethanolic leaf extract at 200 and 400mg/kg of *Solanum melongena* and *Solanum lycopersicum* respectively. 0.25µl of Formalin 5% solution and AITC are administered intra plantar and subcutaneous route to hind paw as inducers of nociceptive pain.

The time taken by animals to show paw flinching behaviour and volume of mercury rise in plethysmograph were observed. The group treated with ethanolic extract at dose 400mg/kg *Solanum melongena* showed maximum response in both the models, indicating its activity in neutralizing pain mediators with respect to standard as mention in tables indicating its activity in treating neuropathic pain. The *Solanum melongena* leaf extract at dose of 400mg/kg showed proficient Neuropathic Pain property compared with other extracts and doses that may be due to presence of more flavonoids and phenolic compounds.

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