Anti-Inflammatory activity of seeds extract of *datura stramonium* against carrageenan induced paw edema on albino wistar rats

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Abstract
Datura, a wild plant from solanaceae family is very well known for its poisonous and medicinal properties. It contains a wide variety of phytoconstituents such as alkaloids, essential oils, flavonoids, tannins, steroids, glycosides etc. They are responsible for various medicinal properties. Wide variety of pharmacological activities of different species of datura have been reported so far such as anti-inflammatory, anti-diarrhoeal, hypoglycemic activity, anti-viral activity etc. Daturastramonium has been used for curing various ailments including ulcer, wounds, gout, fever, asthma, toothache etc. The aim of present study is to investigate the anti-inflammatory activity of seed extract of Daturastramonium against carrageenan induced paw edema model in albino wistar rats.

Keywords: Daturastramonium, Phytoconstituents, Hypoglycemic, Solanaceae, Anti-inflammatory activity.

Introduction
Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair.[1]

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Inflammation is not a synonym for infection. Infection describes the interaction between the action of microbial invasion and the reaction of the body's inflammatory response, the two components are considered together when discussing an infection, and the word is used to imply a microbial invasive cause for the observed inflammatory reaction. Inflammation on the other hand describes purely the body's immunovascular response, whatever the cause may be. But because of how often the two are correlated, words ending in the suffix -itis (which refers to inflammation) are sometimes informally described as referring to infection. For example, the word urethritis strictly means only "urethral inflammation", but clinical health care providers usually discuss urethritis as a urethral infection because urethral microbial invasion is the most common cause of urethritis.

Datura has been used in traditional medicine to relieve asthma symptoms and as an analgesic during surgery or bone setting. It is also a powerful hallucinogen and deliriant, which is used entheogenically for the intense visions it produces.[2,3] However, the tropane alkaloids responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.[4-10] The aim of present study is to investigate the anti-inflammatory activity of seed extract of Daturastramonium against carrageenan induced paw edema model in albino wistar rats.

Materials and Methods
Instruments

Table 1: List of Instruments

S. No	Instrument Name	Company
1.	Micropipette	Himedia
2.	Water bath	Scientech
3.	Weighing balance	OHAUS Pioneer PAG 213
4.	Plethysmometer	Orchid Sientific Digital
5.	Homogenizer	REMI, RQ- 127A
6.	Refrigerator	Whirlpool

Chemicals

Table 2: List of Chemicals

S. No.	Chemical Name	Company
1	Carrageenan	Merck
2	Ninhydrin	Merck
3	Nitric acid	Merck
4	Petroleum ether	Merck
5	Pyridine	Merck
6	Sodium dihydrogen phosphate	Merck
7	Sulphuric Acid	Merck
8	Nacl	Merck

9	Ethanol	Merck
10	Hcl	Merck

Glasswares

1. Test tube
2. Beaker
3. Conical Flask
4. Glass rod
5. Syringe
6. Feeding needle

Collection and authentication of thevetia peruviana seeds

Fresh, well developed *Datura stramonium*seeds were collected from the different places of Bhopal (M.P.). It is abundantly grow in the Bhopal city. It was authenticated by the Taxonomist Dr. Zia-ul-Hasan, Hon'ble HOD, Department of Botany, Saifia Science College, Bhopal. Voucher Specimen (408/Bot/saifia/16) was deposited in the same.

Extraction of datura stramonium seeds

Seeds were dried in the shade and then pulverized. The powder of seeds was extracted with petroleum ether to defattification of material at about 3 days and subsequently with 70% methanol for 5 days by using simple maceration method. The extract was filtered with filter paper and muslin cloth. After filtration, the extract was evaporated to dry by slow heating and continuous stirring on water bath. After the residue extractions, the excess solvents were completely removed to get concentrated, then completely dried and preserved in airtight container under refrigeration.

Animals used

Male and Female Wistar albino rats (180 ± 30 g) were used. They were grouped as four animals per cage under standard laboratory conditions at relative humidity of 30.7% at 22 ± 2 °C with 12 h light/dark cycle. The animals were maintained under standard nutritional and environmental conditions throughout the experiment. The experimental protocol was approved by Institutional Animals Ethics Committee Clearance 1238/A/08/CPCSEA.

Materials and Methods

Solubility testing of Plant sample

The solubility of seeds extract was determined by dissolving the sample in water, DMSO, methanol, ethyl acetate, acetone, chloroform respectively.

Organoleptic testing

The plant sample was evaluated for its organoleptic characteristics such as odour, taste, consistency, thickness.

Phytochemical testing

Detailed phytochemical testing was performed to identify presence of different phytoconstituents (Kokate CK, Purohit AP et.al,2006).

Test for Carbohydrates

Molish Test

2ml. of aqueous extract was treated with 2 drops of alcoholic α-naphtholsolutionina testtube and then1ml. of concentratedsulphuricacidwas added carefully along the sides of the test tube, formation of violet ring at the junction indicates the presence of carbohydrates.

Fehling's Test

To 1 ml. of aqueous extract, 1 ml. of Fehling A and 1 ml. of Fehling B solution were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

Benedict's Test

Equal volume ofbenedict's reagents and extract were mixed in a test tube and heated in the water bath for 5-10 minutes solution appears green, yellow, or red depending on the amount of reducing sugar present in the test solution which indicates the presence of reducing sugar.

Barfoed's Test

1 ml. of extract and barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes, red colour due to the formation of cupric oxideindicates the presence ofreducing sugar.

Test for proteins and Amino acids

1. **Biuret's Test:** The extract was treated with 1 ml. of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of protein.
2. **Millon's Test:** 3 ml. of extract was added with 5 ml. of millon's reagents. White precipitate formed which on heating turned to brick red, indicating the presence of proteins.
3. **Ninhydrin Test:** 3 ml. of test solution was heated with 3 drops ofninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acid.

Tests for Glycosides

1. **Borntrager's Test:** In 3 ml oftest solution, dilute sulphuric acid was added, boiled for 5 minutes and filtrated to the cold filtrate, equal volume of benzene or chloroform was added and shake it well. The organic solvent layer was separate and ammonia was added to it. Formation of pink to blood red colour indicates the presence of anthraquinone glycosides.
2. **Legal's Test:** 1 ml. of test solution dissolved in pyridine 1 ml. of sodium nitroprusside solution was added and made alkaline by using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of cardiac glycosides.
3. **Keller- Killiani Test:** To 2 ml. of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride solution were added in atesttube. Add carefully 0.5ml

concentrated sulphuric acidby the side of the test tube. Formation of blue colourintheacetic acid layer indicates the presence of cardiac glycosides.

Tests for Alkaloids

To the extract, dilute hydrochloric acid was added, shake it well and filtered with the filtrate, the following tests were performed.

1. **Mayer's Test:** To 2-3 ml. offiltrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.
2. **Dragendroff's Test:** To 1-2 ml of filtrate, few drops of Dragendroff's reagent were added in a test tube. Formation of red precipitate indicates the presence of alkaloids.
3. **Hager's Test:** To 1-2 ml. of filtrate, few drops of Hager's reagents were added in a test tube. Formation of yellow colour precipitate indicates the presence of alkaloids.
4. **Wagner's Test:** To 1-2 ml. of filtrate, few drops of Wagner's reagents were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins

Froth Test

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Test for Flavonoids

1. **Lead Acetate Test:** The extract was treated with few drops of lead acetate solution. Formationofyellow coloured precipitate indicatesthepresenceofflavonoids.
2. **Alkaline Reagent Test:** The extract was treated with few drops of sodium hydroxide separately in atest tube. Formation of intense yellowcolour, which becomes colorlessonaddition of few drops of dilute acid, indicates presence of flavonoids.
3. **Shinoda Test:** To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise additionofconcentratedhydrochloricacid.Formation of pink colour indicates presence of flavonoids.

Test for Triterpenoids and steroids

1. **Salkowski's Test:** The extract was treated with chloroform and filtered. The filtrate was added with drop of concentrated sulphuric acid, shaken and allowed to stand if lower layer turns red, sterol is present. Presence of golden yellow layer at bottom indicates the presence oftriterpenoids.
2. **Libermann-Burchard Test:** The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled concentrated sulphuric acid was added through the sides of the test tube formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red colour indicate presence of triterpenoids.

Tests for Tannins and Phenolic Compounds

1. **Ferric Chloride Test:** Some amount of extract was dissolved in distilled water, to this solution 2 ml. of 5% ferric chloride solution was added. Formation of blue, green or violet colour indicates presence of phenolic compounds.
2. **Lead Acetate Test:** Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates of phenolic compounds.
3. **Dilute Iodine Solution Test:** To 2-3 ml. of extract, few drops of dilute iodine solution were added. Formation of transient red colour indicates presence of phenolic compounds.
4. **Gelatin Test:** Some quantity of extract dissolved in distilled water. To this solution 2 ml. of 1% gelatin solution containingl0% sodium chloride was added. Developmentofwhiteprecipitateindicatespresenceofphenolic compounds.

Acute oral Toxicity of *Daturastramonium* seeds extract

Procedure

The acute toxic class method set out according to the OECD Guidelines is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. The dose level to be used as the starting dose is selected from one of four levels 5, 50, 300 and 2000 mg/kg body weight. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound - relatedmortality of the animals dosed at one step determined the next step, i.e. whether or not further testing was needed, dosing of three additional animals, with the same dose and or, dosing of three additional animals at the next higher or the next lower dose level. Three animals were used for each step.

Treatment groups of animals

Animals were procured from animal house of NIPS, Bhopal. All experiments were performed with prior permission of IAEC. Animals were randomly divided into following groups.

Table 3: Treatment group of Animals

Group	Dose
Group 1	Served as vehicle control group and treated daily with normal saline water (3 ml, orally) for three days.
Group 2	Served as inducer group and single dose of Carrageenan 1%w/v(0.1ml) was administered on third day.
Group 3	Served as alcoholic extract group (100 mg/kg, orally) treated for three days, a single dose of Carrageenan 1%w/v(0.1ml) was administered on third day, 1 hr after regular extract treatment.

Biostatistical interpretation of experimental data

All the experimental data were statistically analyzed by using SIGMA STAT-3.5 for Windows, 2006 software and expressed as mean ± S.D. The One Way Analysis of Variance (ANOVA) followed by Bonferroni test was used for interpretation of data. The $P<0.001$ was considered as significant.

Results

Physical Examination

Table 4: Physical evaluation of *Thevetia peruviana* flower extract

S. No.	Organoleptic Characteristics	Results
1.	Colour	Dark green
2.	Taste	Acrid
3.	Odour	Pungent
4.	Appearance	Semi-solid
5.	Consistency	Sticky

Solubility tests

Table 5: Solubility of *Datura stramonium*seeds extract in different solvents

S. No.	Solvent	Observation
1.	DMSO	Completely soluble
2.	Distilled water	Insoluble
3.	Ethanol	Sparingly soluble
4.	Petroleum ether	soluble

Phytochemical Screening

Table 6: Phytochemical investigation of *Datura stramonium*seeds extract

S. No.	Experiment	Results	
		EtOH Extract	Pet ether Extract
1. Alkaloids			
1.1	Mayer's reagent test	+ve	-ve
1.2	Wagner's reagent test	+ve	-ve
1.3	Hager's reagent test	+ve	-ve
2. Carbohydrates			
2.1	Molish's test	-ve	-ve
2.2	Barfoed's test	-ve	-ve
3. Test for Reducing Sugar's			
3.1	Fehling's test	-ve	-ve
3.2	Benedict's test	-ve	-ve
4. Flavonoids			
3.1	Alkaline reagent test	+ve	-ve
3.2	Lead acetate test	+ve	-ve
5. Glycosides			
4.1	Borntrager test	+ve	-ve
4.2	Legal's test	+ve	-ve
4.3	Killer- Killiani test	+ve	-ve

6. Tannins and Phenolic compounds			
6.1	Ferric chloride test	+ve	-ve
6.2	Lead Acetate test	+ve	-ve
6.3	Gelatin test	+ve	-ve
7. Saponins			
7.1	Foam test	+ve	+ve
8. Test for Proteins and Amino Acids			
8.1	Ninhydrin test	-ve	-ve
8.2	Biuret test	-ve	-ve
9. Test for Triterpenoids and Steroids			
9.1	Salkowski test	-ve	+ve
9.2	Liebermann-Burchard test	-ve	+ve

Table 7: Comparison between Control and Test groups using readings obtained at 0, 30, 60, 90, 120 min

Remarks	Time	Control	Test	T value
P>0.10	0min	0.666+0	0.666+0	0.00
p>0.10	30min	0.999+0	0.999+0	0.00
p>0.10	60min	0.999+0	0.999+0	0.00
P<0.001	90min	1.277+0.136	0.999+0	5.00
P<0.02	120min	1.221+0.172	0.944+0.136	3.10

Discussion

Plants have been used during the age for cure and treatment of diseases since the start of mankind. Phytotherapy is the use of plant, plant extract or pure chemicals isolated from natural products to treat diseases. Plants have been used to treat diseases such as analgesia, antimicrobial diseases, inflammatory diseases, cancer diseases, schizophrenia, cracked feet, filariasis, caruncles, rheumatoid arthritis, asthma etc. It is evident that the plant has great potentials in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders (Khanzadaet.al, 2013). In present investigation one of the traditionally used herb's anti-inflammatory being assessed against the carraagenan induced paw edema in albino rats.

Firstly, organoleptic characterization of plant extract was performed. Organoleptic evaluations are subjective, sensory judgements. They can involve eyeing, feeling, and taste of the extract to judge its appearance, colour, integrity, texture and flavours. The organoleptic characters of alcoholic extract of *Daturastramonium seeds* extract were found to be dark brown in colour, semisolid, and taste is acrid.

Solubility testing of alcoholic extract of *Daturastramonium* seeds is done mainly to study the ability of the dissolve in different solvents for preparation of aqueous extract for dosing. The alcoholic extract was observed to be dissolved in DMSO & Chloroform.

In the present study, the preliminary phytochemicals test was done on the alcoholic extract of *Daturastramonium*seeds and it is found to be rich in Alkaloids, Glycosides, Saponins, Flavonoids and Tannin compounds.

For the determination of protective effect of Daturastramonium seeds extract against carragenan induced anti-inflammatory.

In vehicle, carragenaan was induced in two different groups one is control and other is test. The carraggenan extract was given at an interval of 0min, 30min, 60min, 90min, 120min. The results of control are 0.666+0, 0.999+0, 0.999+0, 1.277+0.136, 1.221+0.172. and the result of test are 0.666+0, 0.999+0, 0.999+0, 0.999+0, 0.944+0.136 respectively. From the above discussion it has been shown that the extract has shown the anti-inflammatory activity.

Conclusion

Medicinal plants becoming the most important aspect of global health care and formed the basis of health care throughout the world since the earliest day of humanity. In this project we have taken datura stramonium seeds extract to treat carrageenan induced paw edema in albinowistar rat and it has being successfully studied.

Conflict of Interest: None.

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