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Research Article

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Analgesic Activity of *Quisqualis indica*

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Abstract

The aim of the present study was designed to evaluate the analgesic activity of hydro alcoholic extract of *Quisqualis indica* Linn. leaves in wistar rats. The preliminary phytochemical screening of hydroalcoholic extract revealed the presence of alkaloids, carbohydrates, protein and amino acid, Saponins, glycosides, steroids, tannins, flavonoid and phenolic compounds. The peripheral analgesic activity of hydroalcoholic extract of *Quisqualis indica* leaves (100 and 200 mg/kg p.o.) was studied using experimentally induced pain models: Tail flick method using analgesiometer and acetic acid-induced writhing effect in albino wistar rats of either sex. The plant extracts exhibited dose dependant analgesic activity in the all treated groups and the results were compared with that of standard drug (Aspirin 20 mg/kg, p.o.). Interpretation & conclusions: From our study, we concluded that the hydroalcolic extracts of *Quisqualis indica* Linn. leaves (100, 200 mg/kg, p.o.) possesses dose dependent, significant ($P<0.05$) analgesic activity against experimentally induced pain.

Keywords *Quisqualis indica*, Soxhlet Apparatus, Tail Flick Mehtod, Analgesiometer, Writhing Effect

Introduction

Pain is an unpleasant and emotional experience associated with or without actual tissue damage. The pain sensation is described in many ways like sharp, pricking, electric, dull, achae, shooting, cutting, stabbing etc. Often it induces crying and fainting. It is produced by real or potential injury to the body. Pain may be acute or chronic. Acute pain is a sharp pain of short duration with easily identified cause. Chronic pain is the intermittent or constant pain with different intensities. It lasts for longer periods. It is somewhat difficult to treat chronic pain and it needs professional expert care [1].

Pain sensation various parts of body is carried to brain by different pathways such as pathway from skin and deeper structures, pathway from face, pathway from viscera and pathway from pelvic region.

Neurotransmitters involved in pain sensation are glutamate and substance P are the neurotransmitters secreted by pain nerve endings. The A δ afferent fibers which transmit impulses of fast pain secrete glutamate. C type fibers which transmit impulses of slow pain secrete substance P [2-3].

Material and Methods

Plant Material Authentication

The mature green leaves of *Quisqualis indica* Linn were collected in the morning locally from Jaipur, Rajasthan, India, in the month of August 2014. The plant was identified and authenticated by the Botanist, from the Department of Botany, University of Rajasthan, Jaipur, India (Authentication No.- RUBL211429).

Pharmacognostic Studies

Under this Macroscopy**,** Microscopy, Quantitative Microscopy, Fluorescence Analysis studies of plant's were done.

Extraction and Preparation of Plant Extract

A. Successive Solvent Extraction

Leaves of the plant *Quisqualis indica Linn*. were shade dried. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in Soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. Deffated marc of drug was subjected to extraction with chloroform in a Soxhlet apparatus. The extraction was continued for a period of 48 hours. The extract was then concentrated and finally dried to a constant weight. Marc obtained after chloroform extraction was subjected to extraction with ethyl acetate. The extraction was continued for the period of 48 hours. The extracted was then concentrated and finally dried. Marc obtained after ethyl acetate extraction was subjected to extraction with methanol in Soxhlet apparatus. The extract was then concentrated and finally dried to a constant weight. Lastly the marc obtained was subjected to hot water maceration. The maceration was continued for 24 hours. The aqueous extract was filtered and concentrated.

B. Direct Methanolic Extraction

Two times extraction of the plant is done in the gap of 15 days. It is done by the Soxhlet Apparatus. Hydroalcohol [Methanol and water (70:30)] is used as a solvent. Plant material was collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant material was then shade dried and pulverized in a hand mill followed by sieving (sieve no. 40) to obtain coarse powder. About 180 gm of dry powder was extracted with petroleum ether (40-60 °C) and hydroalcohol for 48 hr in soxhlet extractor. The hydroalcoholic extract was filtered, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The final obtained extract was weighed; percentage yield was calculated and stored in a cool place.

Phytochemical Screening

Phytochemical studies of the hydroalcoholic *extract of Quisqualis indica* was performed for major classes of constituents [4].

Table 1: Table of Phytochemical Screening

Contents	Test	Observation	Results
Alkaloids	Powered drug + Mayer's reagent	No Yellow precipitate formed	Absent
Glycosides	Powered drug + Dilute KOH	Absence of red color	Absent
Sterols	Powered drug + Acetic anhydride	Absence of blue-green color	Absent
Protein	Powdered drug+ 4% NaOH + 1% $CuSO_4$ (Biuret Test)	No Pink color	Absence
Saponin	Drug solution + water and shake (Froth Test)	stable froth be noted	Present
Carbohydrate	Drug solution + Molisch's reagent (Molisch's test)	purple to violet color ring appeared at the junction	Present
Flavanoids	Drug solution + Lead acetate	Color change	Present

Experimental animals

The experimental study was carried out in male wistar rats weighing 150-200 g body weight and the experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Institute of Biomedical and Industrial Research, Jaipur, India (1737/PO/Rc/S/14/ CPCSEA) as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Throughout the experiment, the animals were housed, four animal per cage, maintained at ambient temperature of (25º±2); 30-60% humidity, under 12 hr. light-dark cycle. They were fed with standard pellet diet and water *ad libitum*. The animals were habituated to laboratory conditions for 48 hr. prior to the experimental protocol to minimize any non specific stress.

Acute Toxicity Study and Dose Selection

The Purpose of acute oral toxicity study was to provide information on health hazards likely to arise from a short-term exposure to *Quisqualis indica Linn.* extract by the oral route. An Acute oral toxicity test was conducted with

rats to determine the potential for *Quisqualis indica Linn.* extract to produce toxicity from a single dose via the oral route. Based on the results of this study, the single oral dose of the test substance is greater than 1500 mg/kg.

Animals: Six (3 males and 3 females) healthy young albino wistar rats of Body weight: 150-180 g were used from the Institute of Biomedical and Industrial Research for the study.

Method: **A)** Husbandry:

Housing: Each group was housed in Plastic caging.

Animal Room: Temperature range: 20-25 °C

Photo-period: 12 hrs. dark / light cycle.

Acclimation Period: 15 days

Food & Wate: Food and filtered tap water was supplied *ad libitum* by an automatic water dispensing bottle.

B) Identification:

Cage: Each cage was identified with a cage-card indicating the study number and identification and sex of animals.

Animals: A mark of different colors (For male: Black, Green, Blue; For female: Pink, orange, Red) were given to each rat. These colors constituted unique identification.

Procedures: The acute oral toxicity study was carried out according to OECD 423 guidelines. Referring to old research papers and to avoid unnecessary harm and loss of animals on repetitive work, this was opted that only one group of animals should be subjected to the drug dose. Six albino rats were grouped and an oral dose of 1500 mg/kg of body weight of plant extract was administered.

A. Selection of animals: Prior to dosing, a group of animals was fasted for approximately 17 hours by removing feed from their cages. During the fasting period, the rats were examined for health and weight (initial). Six (3 male and 3 female) healthy rats were selected for test.

B. Dose calculation: Doses were calculated based on the initial body weight.

Dosing: Each animal received 1,500 mg/kg of the test-substance, by stomach intubation. After administration, each animal was returned its designated cage. Feed was replaced approximately 3 hours after dosing. The day of administration was considered day-zero of the study.

A. Body weight: Individual body weight of the animals were recorded prior to test substance administration and again on day 7 and 14.

B. Cage Side Observations: The animals were observed for mortality, signs of gross toxicity and behavioral changes at 1 and 3 hours post-dosing and at least once daily there after 14 days.

Observations included gross evaluation of skin and fur, eyes, respiration, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and comma. It was found that the extract has produced significant toxicity up to the dose of 1500 mg/kg. Thus the extract was highly tolerable up to 1500 mg/kg.

Analgesic activity was determined using following methods

a) Tail flick method

The animals were weighed and appropriately marked. After 15 minutes of drug administration to the animals, the basal reaction time was taken by placing the tip (last 1-2cm) of the tail of animals on the radiant heat source. The tail-withdrawal from the heat (flicking response) was taken as an end point. Normally mouse withdraws its tail within 3-5sec. A cut off period of 10-12 sec was observed to prevent damage to the tail.

The test drug was administered to each group animal and notes the reaction time at the 15, 30, 45, and 60 minutes. As the reaction time reaches 10 sec it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage.

Experimental Design

Four groups were designed, each group comprised of six wistar rats (180–200 g).

Group I	Control group (2% gum acacia)
Group II	Test drug hydroalcoholic extract of *Quisqualis indica Linn.* (100 mg/kg) was administered.
Group III	Test drug hydroalcoholic extract of *Quisqualis indica Linn.* (200 mg/kg) was administered.
Group IV	Aspirin (20 mg/kg, orally) will administered.

b) Acetic-Acid Induced Writhing Effect

Analgesic activity was evaluated on the acetic acid-induced writhing according to Koster et al. (1959). albino rats were divided into four groups of six animals each. The animals were pretreated with test drug (100 and 200 mg/kg, p.o.) or aspirin (20 mg/kg, p.o.) used as a standard drug, 15 min. prior to intraperitoneal injection of 1% (v/v) acetic acid (0.1 ml/10 g). Five minutes after the intraperitoneal injection of acetic acid, the number of writhing during the following 30 min was counted. Control mice received 2% gum acacia (2 ml/kg).

Experimental Design

Four groups were designed, each group comprised of six wistar rats (180–200 g).

Group I	Control group (2% gum acacia)
Group II	Test drug hydroalcoholic extract of *Quisqualis indica Linn.* (100 mg/kg) was administered.
Group III	Test drug hydroalcoholic extract of *Quisqualis indica Linn.* (200 mg/kg) was administered.
Group IV	Aspirin (20 mg/Kg, orally) was administered.

Statistical analysis

All the data was expressed as ± S.E.M. (standard mean error) statistical analysis was performed using Graph Pad 3 Prism software.

Results

Table 2: Identification of Morphological Feature of *Quisqualis indica* Linn. Leaf

S. No.	Features	Observation
1.	Colour (Upper surface)	Dark green color
2.	Odour	Characteristic
3.	Taste	Tasteless
4.	Shape	Ellipticle
5.	Size	7-12cm

Table 3: Botanical Evaluation of *Quisqualis indica* Linn. Leaf

S. No.	Leaf Portion	Observation
1	Apex	Acuminate
2	Margin	Entire
3	Shape	Ellipticle
4	Colour	Green
5	Leaf base	Cordate

Table 4: Transverse Section of *Quisqualis indica* Linn. Leaf

S. No.	Features	Observation
1.	Trichomes	Present both glandular and covering
2.	Upper epidermis	Present
3.	Midrib	Hypodermis is made up of collenchymas
4.	Lamina	After epidermis collenchyma is present

Table 5: Powder Microscopy of *Quisqualis indica* Linn. Leaf

S. No.	Feature	Observation
1	Nature	Coarse powder
2	Colour	Light green
3	Odour	Characteristic
4	Taste	Slight bitter
5	Epidermal cell	Present
6	Stomata	Present (Anomocytic)
7	Fibres	Present
8	Starch grain	Present
9	Calcium oxalate crystals	Present

Table 6: Analysis of Powdered Drug through Naked Eye

Reagents	Colour observed
Powder as such	Fade green
Powder + conc. HCl	Green
Powder + Conc. HNO_3	Brown
Powder + Conc. H_2SO_4	Dark brown
Powder + Glacial acetic acid	Green
Powder + 5 % NaOH	Brownish green
Powder + Picric acid (saturated Aq. Solution)	Yellowish green
Powder + Ammonia	Brownish green

Table 7: Fluorescence Analysis of Powder Drug

Chemical	Fluorescence Observed
Powder as such	Green
Powder + 1N NaOH in methanol	No fluorescence
Powder + 1N NaOH in water	Green
Powder + 50 % HCl	Brown
Powder + 50 % H_2SO_4	Green
Powder + Petroleum ether	Green
Powder + chloroform	Black
Powder + picric acid	Brown

Table 8: % Yield of Extracted Drug

Quantity of crude drug taken for extraction (g)	Method used for extraction	Solvent used	Quantity of extracted drug (g)	% yield (w/w)
180	Hot extraction	Hydroalcoholic (Methanol : water) (70 : 30)	21.2	11.78

Table 9: Phytochemical Analysis of The Hydroalcoholic Extract of Leaves of *Quisqualis indica* Linn.

Constituents	Tests	Hydroalcoholic Extract
1. Alkaloids	Dragendorff's	-
	Hagers	++
2. Glycosides	Legal test	++

3. Carbohydrates	Molish test	+
4. Protein	Biuret test	-
	Copper sulphate test	++
5. Flavanoid	Lead acetate test	++
6. Amine	Lead acetate test	++

Key: ++ = Highly present, + = faintly present, - = absent.

Analgesic Activity by Tail Flick Method:

Table 10: Analgesic effect of Hydroalcoholic extract of *Quisqualis indica* on Wistar rats's using Tail flick method

Group	Drug treatment	Dose	Reaction Time (Sec.)			
			15 min	**30 min**	**45 min**	**60 min**
I	Control (2% Gum Acacia)	2 ml/kg	4.6 Sec ± 0.42	4.8 Sec ± 0.30	5.6 Sec ± 0.33	5.8 Sec ± 0.60
II	Test Drug-1	100 mg/kg	7.3 Sec ± 0.55[a]	6.5 Sec ± 0.34[b]	7.1 Sec ± 0.6[b]	6.6 Sec ± 0.33
III	Test Drug-2	200 mg/kg	6.6 Sec ± 0.42[b]	7.8 Sec ± 0.30[c]	7 Sec ± 0.35[b]	8.5Sec ± 0.21[b]
IV	Standard (Aspirin)	20 mg/kg	7.5 Sec ± 0.52[a]	7.6 Sec ± 0.40[c]	8 Sec ± 0.25[b]	8.3 Sec ± 0.33[b]

Each group N=6 represents mean ± SEM, a = P < 0.01 Vs Control group, b = P < 0.05 Vs Control group, c = P< 0.001 Vs Control group

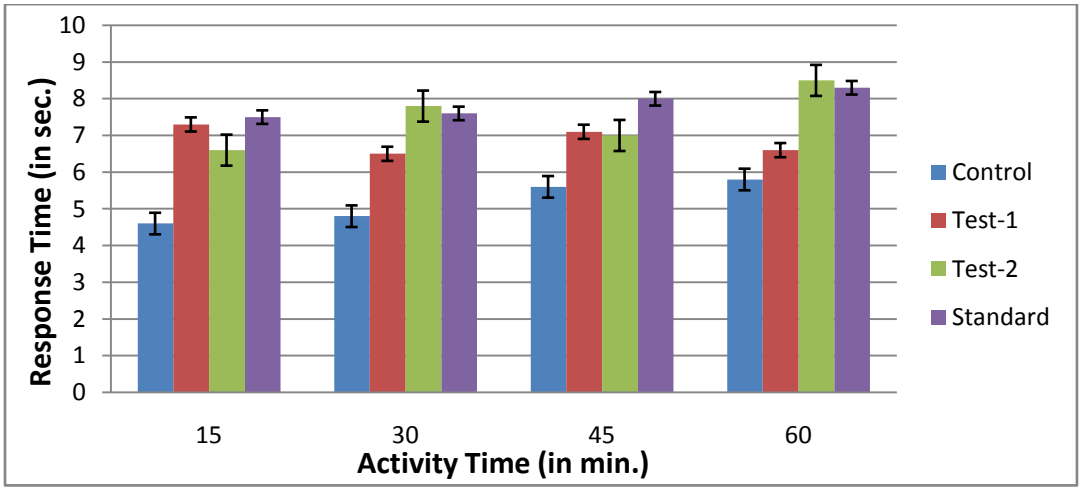

Figure 1: Graphical presentation of Analgesic activity by Tail flick method

The result showed a significant increase (on an average about 40 to 50%) in tail flick time i.e., an increase in the latency period of the extract treated animals as compared to the control group. The findings suggested that hydroalcoholic extract of *Quisqualis indica* possess analgesic activity. The crude hydroalcoholic extract was used in two doses of 100 mg / kg and 200 mg / kg of tested animals under test (Group II and Group III).

Test drug-I (100 mg/Kg) showed significant analgesic effect at 15 minutes (7.3 Sec ± 0.55), 30 minutes (6.5 Sec ± 0.34) and 45 minutes (7.1 Sec ± 0.6). However in Test drug-II (200 mg/Kg) produced significant results at 15 minutes (6.6 Sec ± 0.42), 30 minutes (7.8 Sec ± 0.30), 45 minutes (7 Sec ± 0.35) and 60 minutes (8.5 Sec ± 0.21). The results of the extract treated animals, when compared with control group produced significant results. Standard drug (Aspirin) gave significantly increased tail flicking of rats at in all observed time interval.

The graphical representation of analgesic activity by tail flick method between the response time and activity time on the animals using hydroalcoholic extract has been presented in Figure 1.

Analgesic Activity by Acetic-Acid Induced Writhing Effect

Table 11: Analgesic effect of Hydroalcoholic extract of *Quisqualis indica* on Wistar rats's using Acetic-Acid Induced Writhing Effect method

Group	Drug Treatment	Dose	Number of Writhing	% Inhibition
I	Control (2% Gum Acacia)	2 ml/kg	31.2 ± 0.86	---
II	Test Drug-1	100 mg/kg	23.4 ± 0.50*	25
III	Test Drug-2	200 mg/kg	20.8 ± 0.37*	33.33
IV	Standard (Aspirin)	20 mg/kg	15.8 ± 0.91*	49.35

n = 6. The observations are mean ± S.E.M. * P < 0.05, as compared to control. (ANOVA followed by Dunnett's test)

The result indicate that all the test and standard drugs significantly ($p<0.001$) reduce the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. (Table-11)

As all the drugs are standard analgesics, by applying Student Newman-Keuls test, it was shown that no significant difference between the tests and standard. The standard drug exhibited a writhing inhibition percentage of 49.35%, test drug-1 (25%) and test drug-2 (33.33%) as comparison to control group.

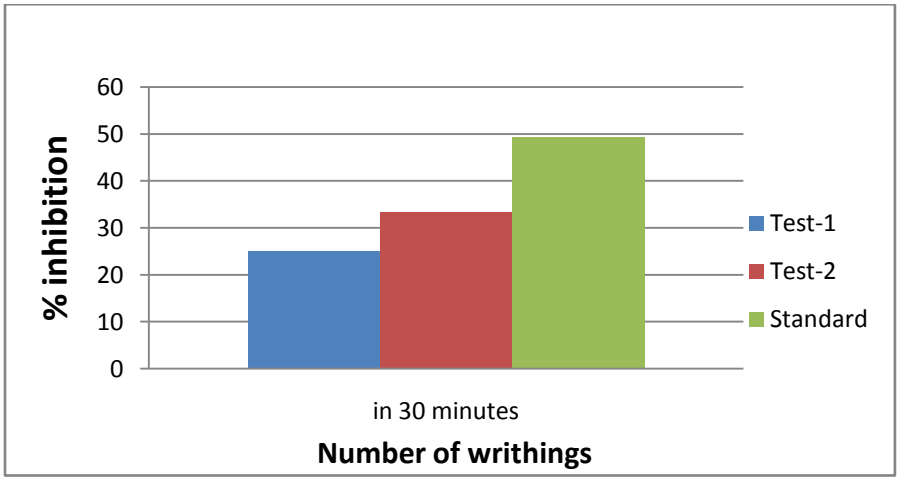

Figure 2: Graphical presentation of Analgesic activity by Acetic-Acid Induced Writhing Effect

Discussion

Development of analgesia is not a complicated process. It can be easily produced with the help of chemicals (Acetic-Acid) and instruments (Analgesiometer). Animal tests of analgesic drugs commonly measure nociception and involve testing the reaction of an animal to painful stimuli [5]. In the present study the thermal test was selected because of several advantages including the sensitivity to strong analgesics and limited tissue damage. The hot plate and tail flick method involve spinal reflexes and is regarded as one of the most suitable methods for studying the involvement of centrally acting analgesics [6]. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase [7]. An increase in reaction time is generally considered as an important parameter of analgesic activity in heat conduction method. In these models, increase in stress tolerance capacity of the animals indicates the possible involvement of a higher centre [8]. It is thought that the analgesic effect of *Quisqualis indica Linn.* seen in this study may involve central activity. The result of this study proved the uses of this plant in folklore medicine for the management of pain.

Conclusion

The hydroalcolic extracts of *Quisqualis indica Linn.* leaves (100, 200 mg/kg, p.o.) possesses dose dependent, significant ($P<0.05$) analgesic activity against stimuli in the tested animals.

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