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Evaluation of Antipyretic and Analgesic Activity of *Ipomoea Quamoclit*

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Abstract
Pharmacological studies were conducted with ipomoea quamoclit extract the experimental animals for evaluating the analgesic, and antipyretic activities. In the analgesic test, ipomoea quamoclit elicited inhibitory intensity on acetic acid-induced writhing response and on the late phase of formalin test but possessed only a weak effect on the tail-flick response and on the early phase of formalin test. The ipomoea quamoclit extract also elicited antipyretic in animals. In addition, ipomoea quamoclit extract showed an anti-antipyretic activities effect when tested in ethyl phenylpropiolate (EPP) and arachidonic acid (AA)-induced.

Keywords: ipomoea quamoclit, antipyretic, analgesic

Introduction

Background information

Fever refers to an increase in body temperature beyond the regulatory set point of 36.5 -37.5 ºC (98- 100ºF). This increase in temperature triggers muscle tone and shivering. Fever signifies several illnesses. Symptoms of fever include sweating, chills, a sensation of cold and other subjective sensations. The absence of these symptoms when the temperature is high can be a pointer to a serious illness [1]. Fever may be caused by infections caused by parasites, viruses, bacteria and immune reactions (including defects in collagen, immunological abnormalities and acquired immunodeficiency [2]. Fever can also be a result of the destruction of tissues during trauma, local necrosis (infarction), an inflammatory reaction in tissues and vessels (flebitis, arthritis), pulmonary infarction, and rhabdomyolysis [3].

In humans, temperature regulation is controlled by the thermoregulatory center in the hypothalamus [4]. The input to the hypothalamus comes from peripheral as well as central thermo-receptors. Research has revealed neural substrates for thermoregulatory control [5]. The peripheral and central thermoreceptors situated beneath the skin are of two subtypes namely those that respond to cold and those responding to warmth [6]. The warm central thermo receptors are located in the hypothalamus, spinal cord, viscera, and great veins, which are more numerous than cold thermo-receptors [7].

Pain is an unpleasant sensory affliction and emotional experience usually associated with actual or potential tissue damage or described in terms of such damage [8]. Pain serves as a warning signal against disturbances. Pain is aimed at protecting the organism but often leads to discomfort [9]. We have two types of pain nociceptive and pathological pain. Nociceptive pain is also known as acute pain, which usually accompanies noxious stimuli warning of impending tissue damage [10]. Pathological pain, according to its cause, is divided into inflammatory pain and neuropathic pain, both belong to chronic pain [11]. Chronic pain completes the list of largest medical health problems in countries with many low-income cadres of people [12]. Treatment of pain and related problems calls for a good understanding of how pain signals are initially interpreted and subsequently transmitted and perpetuated [13].

Antinociceptives such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) have many side effects [14]. Pain studies, treatment and management have made important progress now, but remain underestimated and poorly managed mostly in developing countries [15]. This leads to pain among hospitalized patients is extensive, and significantly more [16].

Materials and Methods

Collection and preparation of plant materials

Fresh plant aerial part of the selected plant was collected from Ranga Reddy District, Hyderabad, and Telangana. These plants are believed by the locals to have medicinal value against various ailments. The plant materials were identified by Dr. Madhaban Chetty, Asst. Professor, Sri Venkateswar University, Tirupati, Andhra Pradeshthe collected plant part was first washed 2−3 times using tap water, to remove adherent particles and dried under shade for com. Preparation of plant extracts was carried out using a protocol as described by Nostro *et al*. (2000) [17]. The dried material was ground to a fine powder using a grinder and passed through a mesh sieve. The powdered materials were kept at room temperature away from direct sunlight in closed dry khaki paper bags.

Extraction

The coarse powder of aerial *Ipomoea quamoclit* was packed tightly in the soxhlet apparatus and extracted with ethanol for 72 hours with occasional shacking maintained at 60ºc throughtout the extraction process. The extract was concentrated to of its original volume by evaporation. The resulting ethanolic extract of aerial *Ipomoea quamoclit* was subjected to phytochemical study.

Procedure:

The animals were divided into two groups and each group consisted of five mice. The defined or fixed dose level of extracts (2000 mg/kg) was given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death.

Antipyretic

Experimental animals

Male Swiss albino rats were used in this study. The rats were aged between 2 and 3 months and with an average weight of 150 grams. The animals were obtained from our institution animal house. They were kept in approved polyethylene cages at room temperature (25±2°C) with 40 to 60 % humidity and 12h dark hours and 12h light cycle. They were provided with standard diet *ad libitum* and water [18].

Preparation of treatment doses

The choice of doses used in this study was arrived at after oral acute toxicity test. The different treatment doses used in this study were prepared as follows, 25, 50, 100, 100, 150, 200 and 250mg/kg body weight dose level, were prepared by dissolving 0.005g, 0.01g, 0.02g, 0.03g, 0.04g and 0.05g of the extract respectively in 0.3ml of 3% DMSO and0.7ml of normal saline was added. To prepare 100mg/kg body weight aspirin each rat needed 13mg of the drug dissolved in 0.5ml of normal saline. Therefore, to prepare a larger volume of the drug, 0.5 g of aspirin was dissolved in 19.23ml of normal saline. All the extracts and solutions administered were freshly prepared.

Experimental design

Experimental rats were splitinto six groups of five animals each (n = 5).

Group I (normal control) comprised normal rats that were administered with3% DMSO.

Group II (negative control) comprised rats that had been inducedwith pyrexia using 20% turpentine. They were administered with 3% DMSO.

Group III (positive control) comprised turpentine-induced pyretic rats that wereadministered with aspirin (100mg/kg bw).

Group IV comprised of turpentine induced pyretic ratsthat were administered with extract dose of 150mg/kg bw.

Group Vcomprised turpentine induced pyretic rats that were administered with extractdose of 200mg/kg bw

GroupVI comprised of turpentine induced pyreticrats that were administered with extract dose of 250mg/kg body weight.

The body temperature of rats in all the groups was taken after fever induction and at hourly intervals following administration of treatments for four hours[160]. Approximately 3cm of a well-lubricated digital thermometer (thermistor probe®) was inserted into the anal region of the rats to measure the rectal temperature. The thermistor animals in the experimental group were taken using both types of thermometers and compared.

The thermostor probe® was first quantified against a mercury thermometer, where temperatures of the animals in the experimental groups were recorded using both thermometers and compared. The baseline/initial mean rectal temperature was calculated by measuring the rectal temperature of rats at fifteen minutes intervals for 1 hour before the induction of fever.

The rectal temperatures of rats were measured and recorded at hourly intervals for 4 hours after the administration of different treatments. The rats whose rectal temperatures rose by one degree Celsius one hour after intraperitoneal injection of turpentine (20mg/kg bw) were termed pyretic and were used for the studies[161]. The difference in rectal temperatures before and after treatments was obtained and the % inhibition in the rectal temperature computed according to the formula as described by Hukkeri*et al.*, 2006; Yemitan and Adeyemi, 2017 [19, 20].

$$\%\ inhibitionofpyrexia = \frac{B - C_n}{B} \times 100$$

Where,

B - Rectal temperature at one hour following turpentine injection

Cn - Rectal temperature after treatments.

Analgesic Activity

Preparation of treatment doses

The preparation of extract doses was done as per the procedure described in antipyretic activity. To prepare 2.5% formalin, 97.5 ml of distilled water was added to 2.5 ml of formalin. To prepare diclofenac.

Experimental animals

Swiss albino mice of both sexes aging between 5-6 weeks of approximately 20gwere used to assess for the analgesic activities of the extracts. The animals were obtained from our institution animal house. They were kept in approved polyethylene cages at room temperature (25±2°C) with 40 to 60 % humidity and 12h dark hours and 12h light cycle. They were provided with standard diet *ad libitum* and water [18]. Swiss albino mice were randomly allocated to six groups of 5 mice (n=5).

Experimental design

A completely randomized experimental design was adopted in this study as described in antipyretic study. Each mouse received treatment as follows;

Group I: (normal control group) comprised normal mice that received 0.01ml of 2.5% formalin.

Group II: (negative control) received 3% DMSO.

Group III: (positive control) received 0.1ml of diclofenac at 15mg/kg body weight and after thirty minutes were administered with 2.5% of 0.01ml formalin as the paininducing agent.

Group IV: Comprised mice that received 150mg/kg body weight of the plant extract and thirty minutes later administered with 2.5 % formalin.

Group V: Comprised mice that received 200mg/kg body weight of the plant extract and thirty minutes later administered with 2.5% formalin.

Group VI: Comprised of mice that received 250 mg/kg body weight of the plant extract and thirty minutes later administered with 2.5% formalin.

The formalin-induced pain was carried out as described by Hunskaar and Hole (1985),[164] where all the animals received 0.1ml of treatments intraperitoneally and 30 minutes later injected with 0.01ml of formalin (2.5%) in the left hind paw to generate pain behavior of shaking, licking, biting and lifting[165,166].

The time taken a licking, shaking, biting or lifting of hind paw induced with pain was measured and recorded[165]. The experimentation of Swiss albino mice was done inside a transparent Plexiglas chamber with a mirror put at the side of the chamber to provide a clear observation of the animals being experimented. Two phases of intensive pain behaviors were determined and recorded singly. The early phase was measured and recorded between zero and the fifth minute while the second phase (late phase) measured and recorded between the fifteenth and thirtieth minute. The percentage of pain inhibition was computed utilizing the following formula.

$$\%\ of pain inhibition = \frac{C-T}{C} \times 100$$

Where,

C = Each phase vehicle control group value

T = Each phase treated group value

Statistical analysis

Data on pain was obtained, recorded and entered into Microsoft Excel broadsheet. It was cleaned and then transferred for statistical analysis in Minitab statistical software (version 17.0). The data were subjected to descriptive statistics and expressed as mean ± SEM. An inferential statistic one-way ANOVA was applied to analyze for statistical variation among various sets of treatment groups accompanied by Tukey's post hoc test for mean separations and comparison. Antinociceptive effects of the two plant extracts were carried out using unpaired student t-tested. The confidence level was set at 99.5% ($p \leq 0.005$).

Result and Discussion

Extract nature and extractive value

The ethanolic extract of the aerial part of the plant Ipomoea quamoclit was obtained as dark green in colour and the percentage yield was found to be 9.60%.

Pharmacological evaluation

Antipyretic activity

The extract of *Ipomoea quamoclit*generally exhibited *in vivo* antipyretic activities in rats, which was evidenced by a reduction in rectal temperature against turpentineinduced fever. After one hour of treatment, the groups of Swiss albino rats that received aspirin (100mg/kg body weight) and the extract doses of 150, 200 and 250 mg/kg bw lowered the rectal temperature to 97.05%, 97.11%, 96.55% and 96.69% respectively (Table 6.2).

The *Ipomoea quamoclit*extract dose of 200 caused the highest antipyretic activity, which reduced pyrexia by 2.43% in the first hour. This change was higher than that caused by the reference drug, aspirin, which reduced pyrexia by 1.93%. However, the effect of aspirin was comparable to that of extracts dose levels of 150, 200 and 250 ($p > 0.005$).

In the 2nd hour, the *Ipomoea quamoclit* extract reduced the elevated rectal temperature in a dose-dependent fashion. At doses of 150, 200 and250, the extract lowered the raised rectal temperature to 95.91%, 95.77% and 95.72% respectively. The antipyretic activities of the leaf extract doses of 150 and 200 were statistically similar and comparable to that of aspirin ($p < 0.005$.

In the 3rd hour post-treatment, the leaf extract doses of 150, 200 and 250 lowered the elevated rectal temperature in rats to 95.35%, 95.12%, 95.39% respectively. Similarly, at this hour the extract showed a dose-independent antipyretic potential. The rats that received the *Ipomoea quamoclit* extract at doses of 150, 200 and 250 exhibited antipyretic activities that were significantly different ($p < 0.005$;). However, the antipyretic activity of aspirin was statistically similar compared to that of the extract at all tested dose levels ($p > 0.005$; T).

In the 4th hour, the *Ipomoea quamoclit* extract reduced raised rectal temperature in a dose-dependent manner. The extract of *Ipomoea quamoclit* doses of 150, 200 and 250 reduced pyrexia to 94.52%, 94.17%, 94.15%, respectively. At this hour, the group that received extract of *Ipomoea quamoclit* at a dose of 250 recorded the highest antipyretic effects, which was higher than that of aspirin. The antipyretic effects of the extract doses of 150, 200 and 250 were not significantly different from each other and were comparable to that of the reference drug, aspirin ($p > 0.005$).

Notably, the animals in the normal control group showed no remarkable change in rectal temperature from zero to the fourth hour ($p > 0.005$). However, the animals in the negative control group had a significant increase in rectal temperature from hour zero to hour three ($p < 0.005$). On the other hand, there was a significant reduction in rectal temperature of rats that were treated with aspirin and *Ipomoea quamoclit* extract at all the doses tested from hour zero to the fourth hour ($p < 0.005$).

Table 1: Antipyretic effects of *Ipomoea quamoclit* on turpentine-induced pyrexia in rat

Group	Treatment	Percentage change in rectal temperatures (ºC)				
		0hr	1hr	2hr	3hr	4hr
Group I	3% DMSO	100.00±0.00	98.87±0.13a (0.11)	100.12±0.22a (-0.16)	98.72±0.13a (0.22)	98.88±0.18a (0.11)
Group II	Turpentine + DMSO	100.00±0.00	100.41±0.06a (-0.42)	100.11±0.10a (-0.57)	100.65±0.18b (-0.68)	100.51±0.12a (-0.52)
Group III	Turpentine + Aspirin	100.00±0.00	97.05±0.07cd (1.93)	96.52±0.15bc (2.45)	95.81±0.12cde (3.18)	94.29±0.16d (4.69)
Group IV	Turpentine + 150 mg/kg bw	100.00±0.00	97.11±0.15cd (1.87)	95.91±0.05cd (3.06)	95.35±0.09e (3.63)	94.52±0.07cd (4.47)
Group V	Turpentine + 200 mg/kg bw	100.00±0.00	96.55±0.10d (2.43)	95.77±0.06cd (3.20)	95.12±0.08e(3.87)	94.17±0.07d (4.80)
Group VI	Turpentine + 250mg/kg bw	100.00±0.00	96.69±0.05d (2.29)	95.72±0.07d (3.27)	95.39±0.05e (3.59)	94.15±0.10d(4.83)

Values are expressed as mean ± SEM for 5 rats per group. Values with different superscript letters are statisticallysignificant (p≤0.005) along the same column. The figures in brackets represent mean % inhibition.

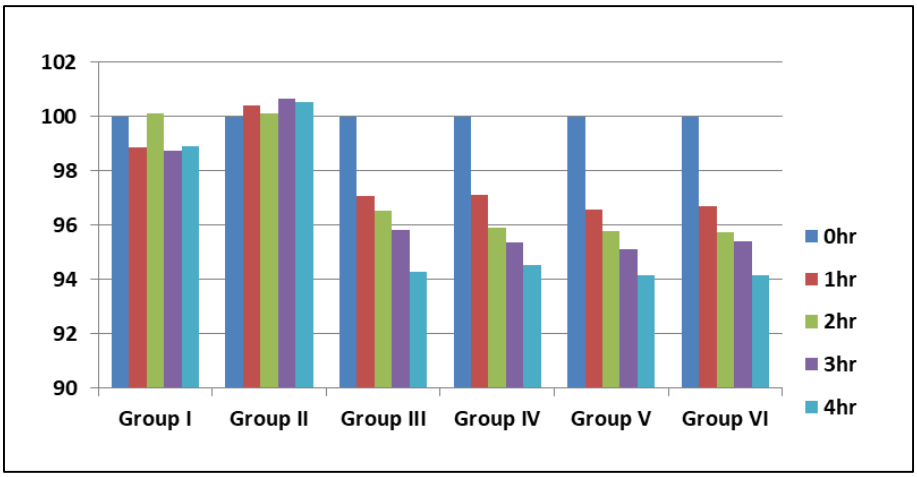

Fig 1: Percentage change in rectal temperatures (ºC)

Analgesic activities

Theantinociceptive activity of aerial part of*Ipomoea quamoclit* extracts was assessed by formalin-induced nociception in Swiss albino mice.They include the early phase which persisted for the first 5 minutes and the latephase which endured between the fifteenth to the thirtieth minute after injection offormalin. The animals that received leaf extract of *E. globulus* revealed analgesicactivity on the formalin-induced nociception in the two phases. This was evidentby a decline in paw shaking, biting, licking and lifting time.

The Swiss albino mice that received aerial part of *Ipomoea quamoclit* extracts at the dose levels of150, 200 and 250 mg/kgbw as well as diclofenac (15mg/kg bodyweight), decreased the paw licking time by 31.71%,29.76%, 30.57% and 31.87% respectively in the early phase. Theanalgesic activity of *Ipomoea quamoclit* extract at the three dosages exhibited a significant difference in the early phase (p <0.005). However, the analgesic effectof diclofenac was statistically insignificant compared to the effect of *Ipomoea quamoclit* at dosages of 150, 200 and 250 in the early phase (p <0.005). The antinociceptive effect of the aerial part of *Ipomoea quamoclit* showed a dose independent response in the early phase.

The mice that were administered with aerial part ethanolic extract of *Ipomoea quamoclit* at the dose levels of 150, 200 and 250 including the aspirin lowered the paw licking time by 90.65%, 94.49%, 98.52% and 98.32% respectively in the late phase.

The analgesic activity of the extract of aerial part of *Ipomoea quamoclit* at the three dosages revealed a significant difference in the late phase (p <0.005;. However, the analgesic activity of the diclofenac was comparable to that of extract of aerial part of *Ipomoea quamoclit* at a dose of 250 in the late phase (p >0.005;. The analgesic effect of the extract of aerial part of *Ipomoea quamoclit* showed a dose dependent response in the late phase. In comparison, the analgesic effect of extract of aerial part of *Ipomoea quamoclit* at all the three dose levels was significantly effective at the late phase compared to the early phase in mice (p <0.005;.

Table 2: nalgesic activity *Ipomoea quamoclit* aerial part extract on formalin-induced pain in mice

Group	Treatment	Early Phase (1-5 min)	Late Phase (15-30 min)
Group I	3% DMSO	0.00±0.00e (0.00)	0.00 ±0.00h (0.00)
Group II	DMSO + Formalin	121.00±1.55a (100.00)	293.40±9.58a (100.00)
Group III	Diclofenac 15mg/kg bw + Formalin	82.77± 1.39d (31.87)	4.00±0.89g (98.32)
Group IV	150mg/kg bw + Formalin	83.01±0.55d (31.71)	26.78±1.16e (90.65)
Group V	200mg/kg bw + Formalin	85.34±1.96d (29.76)	15.39±1.03f (94.49)
group VI	250mg/kg bw + Formalin	84.39±1.78d (30.57)	3.37±0.75g (98.52)

Values are expressed as mean ± SEM for 5 mice. Values with a different superscript letter are statistically significant along the same column by one-way ANOVA followed by Turkey's post hoc test (p≤0.005). The values in brackets represent % pain inhibition.

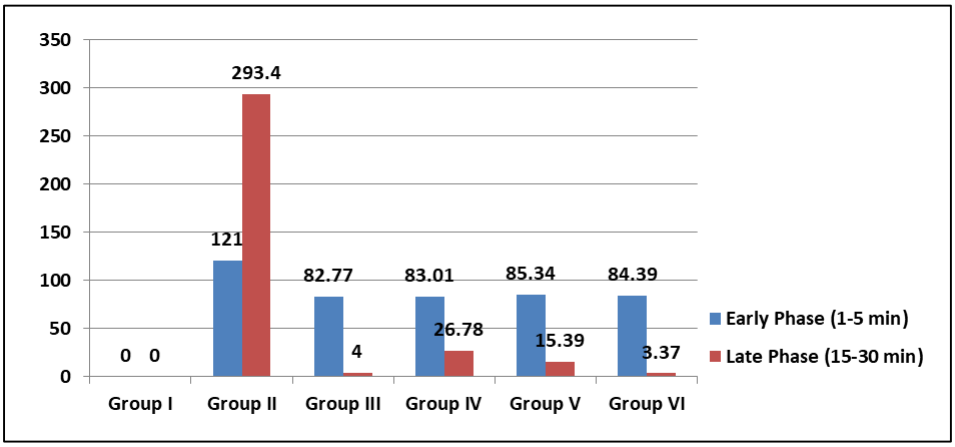

Fig 2: Analgesic activity Ipomoea quamoclit

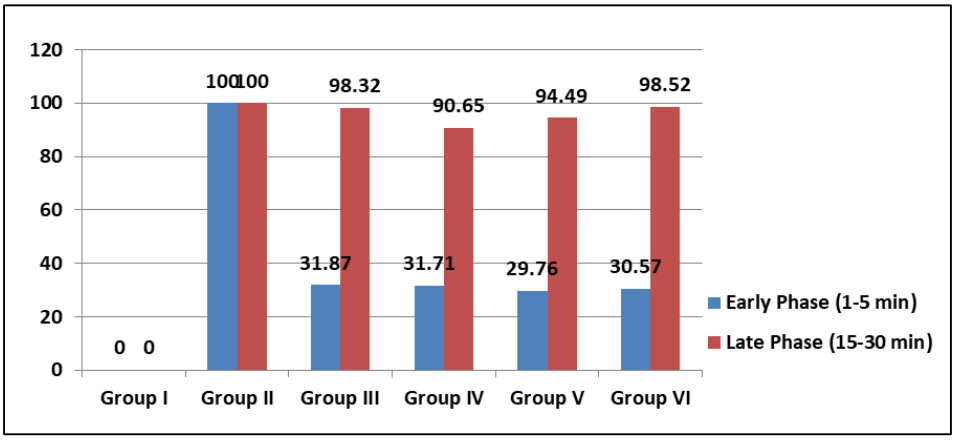

Fig 3: % pain inhibition

The analgesic activities of the extract of aerial part of *Ipomoea quamoclit* exhibited no significant difference at the doses of 150 and 200 in the latephase ($p > 0.005$; Figure 5.4).

Conclusions

This study revealed that the *Ipomoea quamoclit* extracts possess potent antipyretic and analgesic activities. The study also revealed that the effects of plant extract at doses of 200 and 250mg/kg bw were comparable with that of the reference drugs, aspirin and diclofenac. The synergistic and additive effects of the bioactive constituents increase their bioavailability and action on multiple molecular targets thus, the observed activities of *Ipomoea quamoclit* extract is attributed to secondary metabolites they contain. This study, therefore, confirms and supports the use of the studied plant extracts as an alternative and/or complementary remedy against pain and fever. The study also sets the pace for further studies in an effort to develop plant-derived drug compounds for the treatment of pain, fever and inflammation.

From this study, it is concluded that;

1. The ethanolic extract of aerial part of *Ipomoea quamoclit* has several phytochemical agents associated with antipyretic activity in rats.
2. The ethanolic extract of aerial part of *Ipomoea quamoclit* contain phytochemical compounds associated with antinociceptive potential in mice.
3. The ethanolic extract of aerial part of *Ipomoea quamoclit* contain phytochemical compounds associated with anti-inflammatory activity in mice.

Therefore, the research questions formulated in this study were answered in the affirmative and the study objectives were successfully executed and achieved.

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