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Research Article

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Relative Study of Different Fractions of *Ceiba pentandra* by Analgesic Activity

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ABSTRACT

Analgesic effects of the tannin and flavonoid fractions extract of the bark and leaves of Ceiba pentandra Gaertn (Bombacaceae) were recorded in rat. The pain was studied using analgesiometer with help of hot plate and tail flick Methods. Tannin and flavonoid fractions extract of Ceiba pentandra presents in significant analgesic activity. Tannins and Flavonoids present in the extract seem to be responsible for the activity. It is commonly used in anti-bacterial, wound healing, anti-inflammatory activities etc. comparatively checking with tannins and flavonoids fractions extracts shows hyper amount of flavonoids present in leaves, hyper amount of tannins presents in bark.

Keywords: Analgesic effect; Tannin; Flavonoid

INTRODUCTION

Medicinal Plants is having a great capacity to cure the Therapeutic disorders and also treats the Acute and chronic type of disorders. Pharmaceutical active ingredients are isolated from medicinal plants by using different solvents and different extraction methods. Acute toxicity studies are involved for *in vivo* methods before performing the Pharmacological Activities [1-6].

Plant Profile

Botanical source: *Ceiba pentandra*,

Family: Bombacaceae,

Common name: Kapok, *Ceiba*, white silk-cotton tree,

Parts used: Leaves and bark.

Figure 1. *Ceiba pentandra* plant

PHYTOCHEMICAL STUDIES

Collection and Authentication

The plant specimen (leaves) for the proposed study was collected during the month of July 2018 from the garden of Vaageswari College of pharmacy. It was identified and authenticated by Rasingam, scientist incharge of Botanical Survey of India (BSI), Hyderabad. A voucher specimen No. BSI/DRC/2018-2019/Tech/553 has been deposited for further reference, Animal ethical committee no. VCP/cology/006/11/2017.

Extraction of Tannin and Flavonoid Leaf and Bark Fractions

The leaves and bark of *Ceiba pentandra* plant stay shadow desiccated and roughly granulated. Around 300 gm of granulated medicament was distillated with Acetone and Water in ratio of 7:3 by cold steep mechanism. Thereafter 72 hours of steep it was strained. To this extract, Petroleum ether (it is chiefly pre-owned to clear away smooth material in leaf juice) displace by acetone (for discharge of chlorophyll) stay combined in a separating funnel to discard the Chlorophyll. Later ejection of chlorophyll, petroleum ether film was drained. Once more to this extract summate a impregnate solution of sodium chloride (salt water effort to drag water from biotic film to water film) and vitamin-C or Ascorbic Acid (techniques utilized for bioactive mixture separation like flavonoids, tannins and phenolic acids), on the other hand distill the elucidation. To this purified elucidation add Ethyl Acetate firm to separate flavonoids present in solvent. Later moderate agitation of one and the other these firm in a separation funnel, pour off ethyl acetate firm provide the flavonoid. The separation funnel contains solvent film. Afterwards absolute removal, the juice was intense by refine off the firm and then vaporization to drought beneath decreased force applying vacuum flash evaporator through evaporation which allows tannins [7-9] (results will be shown in Tables 1-3 and Figure 2).

PHARMACOLOGICAL STUDIES

Acute Toxicity Study

Aim: Determining the toxicity studies for the given sample of herbal extract using acute toxicity model.

Principle: Determination of acute toxicities is usually and initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The aim of acute toxicity test is to determine the therapeutic index i.e. ratio between the lethal dose and pharmacologically effective dose in the same strain and species.

$$\text{Therapeutic Index}=\frac{Ld\ 50}{Ed\ 50}$$

The greater the index the same for the compounds and vice versa.

Discussion: Data from the acute study inserve as the basis for classification and labeling. Provide initial information on the mode of toxic action of a substance helps to arrive at the dose of a new compound. Help in dose determination in animal study. Helps to determine Ld50 values that provide may in diseases of potential types of drug activity.

Procedure: 3 groups of animals (containing 3 animals per group) by obtained from random breeding from a closedcoloni, because the aim was to discover new and unexpected effects of a in groups of animals of wider variability:

- Group-I received dose of vehicle control,
- Group-II received dose of 2000 mg/kg,
- Group-III received dose of 1 ml/kg.

The following administration, the animals where placed separately in glass cylinder for closed observation and should not be group together same cage because aggregation of mice has been shown the effect the healthy *Ld50* value of some drugs (ex: Amphetamine) the herbal extract was administered orally to Rats that have been fasted for 18 h. The drug administered mice observed continuously for 2 h and then occasional for that 4 h. Finally overnight motility was recorded behaviour of the animal were observed carefully and recorded for the following signs. Increased motor activity, anesthesia, tremors, arching and rolling, chronic cocavulsions, tosis, tonic extentions, lacrimation, exophthalmos, pyloerection, salavation, muscle spasm, opisthotonus, wrighting, hyper esthesia, loss of wrighting reflex, depress, ataxia stimulants, sedation, cyanosis, hypnosis and analgesia [10-14].

Report: No toxic symptoms and mortality is observed in the animals at altered dose.

Determination of acute oral toxicity usually an initial screening step in the assessment and evaluation of the toxic characteristics of all required compounds (results will be shown in Table 4).

Analgesic Activity

Analgesic Activity of Tannins and flavonoids Fraction of Leaves and Bark of *ceibapentandra* have been used as antiinflammatory, antibacterial wound healing activities for the treatment of Analgesic Activity. These are two methods:

- Hotplate Method,
- Radiant Heat Method (Tail Flick Test).

Hot Plate Method

The paws of rodents are highly sensitive to heat at temperatures which do not damage their skin. They usually respond by jumping, withdrawing of paws and licking them. The time required for the onset of these responses in central animals is prolonged by centrally acting analgesics whereas peripheral analgesic and NSAIDs do not affect these responses.

Methodology: Groups of 10 Swiss mice of either sex weighing between 20-25 g are used for each dose. The commercially available Eddy's hot plate consists of an electrically heated surface. The temperature is controlled at 55-56°C. The animals are placed on the hot plate and the latency is recorded before and after 20, 60 and 90 min by following S.C. or Oral administration of the test compounds and the standard drug (Diclofenac sodium injection I.V.) The prolongation of latency times comparing the values before and after the administration of drugs can be

used for comparison using the student's t-test, ED50 values can be calculated using 3 doses of test/standard producing dose dependent increase in the latency. The method has been found suitable for screening centrally acting analgesics. However, it suffers from the drawback that sedatives, muscle relaxants and psychotomimetics can give false positive results [15-17] (results will be shown in Table 5 and Figures 3-5).

Radiant Heat Method (Tail-Flick Test)

The tail flick procedure of D'Amour and Smith (1941) has become standard screening method for the evaluation of analgesics in rats and mice. It has been suitably modified by a number of workers and instruments have been designed for measuring tail flick latencies.

Methodology: Groups of 10 Swiss mice of either sex weighing between 20-25 g are used for each dose. Before the administration of the test or standard drug (Diclofenac sodium injection I.V.) the normal reaction times are determined. The animal is put into a small cylindrical mouse holder with or opening for tail of the rear end. The tail is held gently by the investigator. By opening a shutter, a light beam exerting radiant heat is directed to the proximal third of the tail. For about 6 s are not used for the test. The escape reaction which is the end point of this test is a complex phenomenon and is centrally mediated. In contrast the simple tail flick, used as an end point may be mediated as a spinal reflex. Hence the observation of the escape reaction is regarded as a true assessment of the effect of the drug on the brain. The test compounds and the standard are administered either orally or S.C. These animals are subjected to the some testing procedure after 30, 60, 90 and 120 min. For each individual animal the reaction time is noted. The average values of reaction time after each time interval are calculated and compared with the pre-test value by analysis of mice a very effective for estimating the efficacy and potency of centrally acting analgesics [18-20] (results will be shown in Table 6 and Figure 6).

RESULTS

Phytochemical Screening

Table 1. isolation of tannin and flavonoid leaf and bark fractions

Extract/Fraction	Percentage Yield (%w/w)	Color	Consistency
Leaf and Bark Flavoniods fraction	7.9 and 6.1	Light green	Greasy
Leaf and Bark Tannins fraction	6.5 and 8.1	Brownish	Hard

Table 2. Chemical Tests for Tannins

S.no	Test	Result
1	Ferric Chloride	+
2	Lead Acetate	+

Table 3. Chemical Tests for Flavonoids

S.no	Test	Result
1	Schinoda	+
2	Ferric Chloride	+

Table 4. Acute Toxicity Study

S.No.	Signs	Test Observation	Control Vehicle
1	Increased motor activity	+	-
2	Anaesthesia	-	-
3	Tremors	-	-
4	Arching and Rolling	+	-
5	Clonic convulsions	-	-
6	Ptosis	-	-
7	Tonic extensions	-	-
8	Lacrymation	-	-
9	Exothalamus	-	-
10	Pilo erection	-	-
11	Salivation	-	-
12	Muscles spasm	-	-
13	Opisthotonus	-	-
14	Wrighting	-	-
15	Hyper Esthesia	-	-
16	Loss of light reflux	-	-
17	Depression	-	-
18	Ataxia Stimulation	-	-
19	Sedation	-	-
20	Cyanosis	-	-
21	Hypnosis	-	-
22	Analgesia	+	-

Figures 2. Extraction Procedure

Hot Plate Method

Table 5. Hot plate method result

Drugs	30 min	60 min	90 min	120 min
Control (Normal Saline Solution)	2.5 ± 0.957	3.1 ± 0.68	3 ± 1.154	3.5 ± 0.957
Standard (Diclofenac sodium injection I.V.)	2.5 ± 0.957	3.5 ± 1.70	3.1 ± 1.34	2.8 ± 1.067
Test (T1) (FLF)	2.8 ± 1.067	4 ± 0.68	4.8 ± 0.687	4 ± 0.816
Test (T2) (TLF)	3.6 ± 0.942	3.3 ± 0.74	2.8 ± 1.067	2.16 ± 1.067
Test (T3) (FBF)	2.83 ± 1.067	3.6 ± 0.942	2.8 ± 0.74	2.66 ± 0.74
Test (T4) (TBF)	4.8 ± 1.067	5.3 ± 0.745	4.8 ± 0.68	5.3 ± 0.471

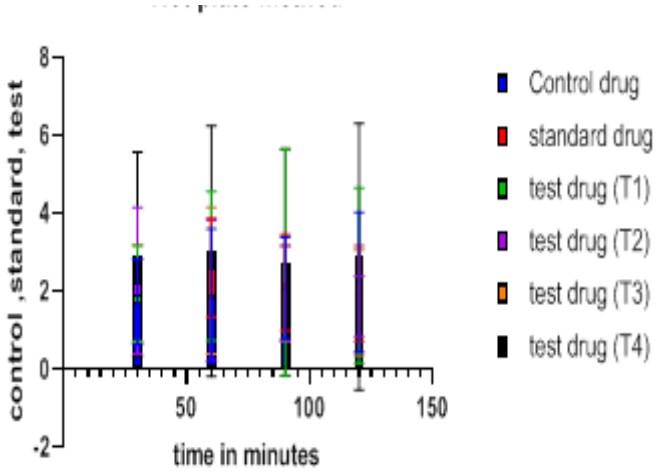

Figure 3. Hot plate method

Tail Flick Method

Table 6. Tail flick method Result

Drugs	30 min	60 min	90 min	120 min
Control (Normal Saline Solution)	2.5 ± 0.957	2.66 ± 1.105	2.83 ± 1.067	2.83 ± 1.067
Standard (Diclofenac sodium injection I.V.)	3.5 ± 0.5	4.3 ± 0.745	4.6 ± 0.471	3.5 ± 0.763
Test (T1) (FLF)	3.66 ± 0.471	4.8 ± 0.687	5 ± 0.577	4.166 ± 0.687
Test (T2) (TLF)	3.1 ± 0.68	3 ± 1.154	3.16 ± 0.89	3.16 ± 0.687
Test (T3) (FBF)	2.16 ± 1.06	2.16 ± 1.06	2.5 ± 1.25	2.33 ± 1.105
Test (T4) (TBF)	4.8 ± 0.89	4.66 ± 1.37	4.8 ± 1.06	4.6 ± 1.067

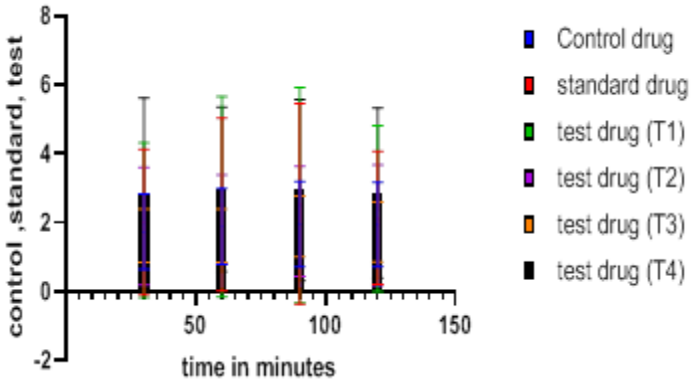

Figure 4. Tail flick method

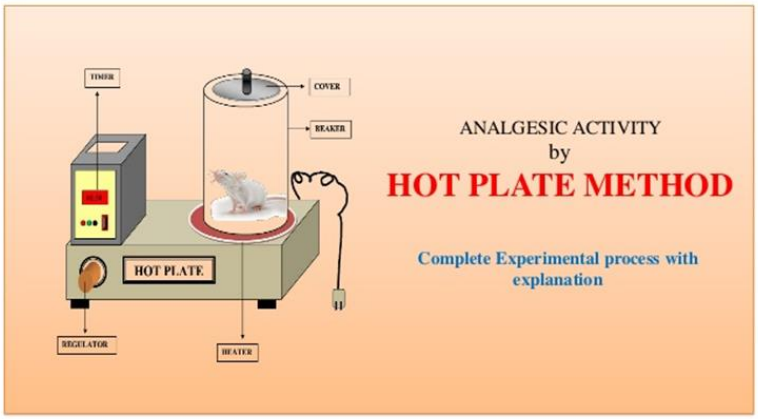

Figure 5. Hot plate method

Figure 6. Tail-flick method

DISCUSSION

The analgesic activity of a drug for thermal pain is tested using Hot Plate and Tail-Flick Analgesiometer in which time of paw licking and tail flick responses are noted respectively. These responses are measured and recorded before and after the drug administration and can be compared with the standard (Diclofenac sodium injection I.V.)

SUMMARY

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness. The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli which include tail-flick and hot plate methods.

CONCLUSION

From this study, it can be concluded that analgesic activity for acute pain at lower dose but higher doses showed an evidence of analgesic activity in experimental conditions and animals we used. The analgesic activity of test drug exhibited at higher doses need to be further evaluated by planning extensive animal experimentation using different animal models.

In conclusion ,the isolated plant leaf fractions contains high amount of flavonoids as the leaves are rich in flavonoid content than tannins and the isolated plant bark fractions contains high amount of tannins as the bark are rich in tannins content than flavonoids.

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