



(RESEARCH ARTICLE)



EVALUATION OF ANALGESIC ACTIVITY OF POPPY SEEDS AND CHAMOMILE IN WISTAR RATS

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ABSTRACT

Pain management is an important field of pharmacological research, due to the restrictions and side effects of the traditional analgesic drugs. The present study was designed to evaluate the analgesic activity of the test compound/extract in Wistar Rat models by standard experimental methods. Healthy adult Wistar rats were divided into different groups like control, standard and test groups. Animals were given normal saline, a standard analgesic drug or various doses of the test substance.^[1]

Analgesic activity was evaluated by using the commonly used experimental models like hot plate method and tail flick method. The reaction time to thermal stimuli was recorded before and after the administration of the treatments at specific time intervals. The results showed a significant increase in pain threshold and reaction time in treated groups as compared to control group indicating significant analgesic potential. The effect was dose dependent and comparable with that of standard drug.^[2]

Keywords: Analgesic activity, Pain management, Wister rats, Reaction time.

1 INTRODUCTION

One of the most common clinical symptoms is pain, an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Analgesic drugs are medications that relieve pain without causing loss of consciousness. The search for effective and safe analgesic agents still remains an important area of pharmacological research as many of the currently available drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are associated with undesirable side effects including gastric irritation, tolerance, dependence and respiratory depression.^[3]

Experimental animal models play a crucial role in the evaluation of analgesic activity and the discovery of new therapeutic agents. Wistar Rat are commonly used laboratory animals in pharmacological studies due to their well-

characterized physiology, easy handling, and predictable responses to pain stimuli. Various experimental methods such as the hot plate test, tail flick test, and writhing test are widely employed to assess central and peripheral analgesic effects in Wistar rats.^[4]

Studies on analgesic activity in Wistar rats are helpful for researchers to determine the efficacy and mechanism of action of synthetic drugs, herbal extracts and novel chemical compounds. These studies provide important pre-clinical evidence of the safety and therapeutic potential of the test substances before clinical trials in humans. Therefore, evaluation of analgesic activity in Wistar rats is an important step in the development of better pain-relieving medications with less side effects and increased efficacy.^[5]

1.1 CHAMOMILE FLOWER

- The flowering plant itself is the biological source of chamomile flowers. There are two primary plant species that provide chamomile flowers:
- *Matricaria recutitather* name for German chamomile

Family: Asteraceae family



Figure 1: Chamomile flower

2 MATERIAL AND METHODS

2.1 METHOD 1: Ethanolic extract of chamomile flower

Plant Material: Chamomile flower (*Matricaria chamomilla*) werw selected for preparation

2.2 Procedure

- Fresh chamomile flowers were picked and washed thoroughly to remove dust and dirt.
- The flowers were shade dried at room temperature for several days so as to avoid degradation of active constituents.
- Dried flowers were powdered by using mechanical grinder to get a coarse powder.
- The powdered material was transferred into a thimble and placed into the Soxhlet apparatus.
- Ethanol was used as the extraction solvent and added to the round bottom flask.
- The extraction was carried out continuously for 6-8hours at controlled heating.
- After extraction completion using whatman filter paper.
- The filtrate was concentrated to get semisolid extract and stored in air tight container for further experiments studies.^[6]



Figure 2: Soxhlet apparatus

2.3 PHYTOCHEMICAL TESTS

2.3.1 Test for Terpenoids

- Tschugaeff test
- A few drops of tschugaeff reagent (picric acid and sodium hydroxide) is added to the test solution
- Observation: Orange brown/ deep yellow
- Inference: Presence of terpenoids



Figure 3: Tschugaeff test

2.3.1.1 Test for Glycosides

- Keller killian test
- Few drops of keller killian reagent (glacial acetic acid and sulphuric acid)
- Observation: Reddish brown colour
- Inference: Presence to glycosides



Figure 4: Keller killian test

2.3.1.2 Test for Flavonoids

- Ferric chloride Test
- Few drops of ferric chloride solution is added to the test solution
- Observation: Green, dark green, brownish green colour
- Inference: Presence to glycosides. [7]



Figure 5: Ferric chloride Test

2.4 POPPY SEEDS

The dried, ripe seeds of the opium poppy plant are the biological source of poppy seeds

FAMILY: Papaveraceae.



Figure 6: Poppy seeds

2.5 METHOH 2- COLD EXTRACTION OF POPPY SEEDS

The cold extraction method or maceration method is a technique for the extraction of active chemical constituents from plant materials by steeping the plant materials in a suitable solvent at room temperature without application of heat. In this process the soluble This method is particularly useful for isolating heat sensitive and volatile compounds from medicinal plants and herbal materials. The most frequently used solvents for cold extraction are ethanol, methanol, water and hydroalcoholic solutions. ^[8]

2.5.1 Collection and Drying

The plant material is collected, cleaned and dried in the shade to remove moisture.

2.5.2 Powder Preparation

The dried material is then ground into a coarse powder using a grinder or mortar and pestle

2.5.3 Soaking (Maceration)

Take a precise quantity of powdered plant material into a clean conical flask or sealed container. Then add enough of the proper solvent to cover the powder entirely.

The container is tightly closed and kept at room temperature for 48–72 hours. The mixture is occasionally shaken to enhance extraction.

2.5.4 Filtration

Once maceration is finished, the mixture is filtered through Whatman filter paper to separate the liquid extract from the solid residue.

2.5.5 Concentration of Extract

The filtrate is concentrated in a rotary evaporator or water bath below 50°C to evaporate the solvent and afford a semi-solid extract.

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2.5.7 Storage

The concentrated extract is collected, weighed, and stored in airtight containers under refrigerated conditions for further pharmacological or phytochemical studies. ^[9]

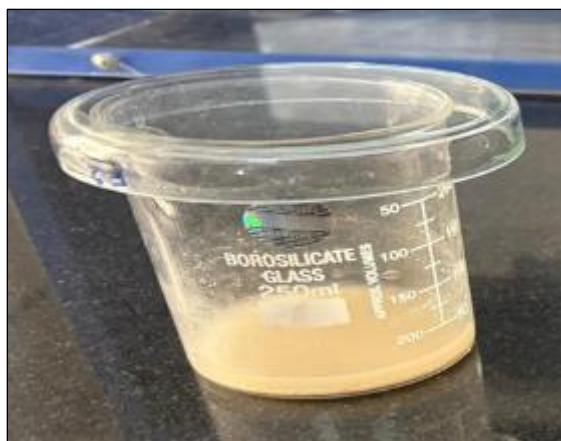


Figure 7: Cold extraction of poppy seeds

2.6 METHOD 3-BANANA PEEL EXTRACT

- BANANA PEEL EXTRACT
- Rinse banana peels with clean water to clean off dirt and impurities.
- Dry them under shade for 2-5 days.
- Grind dried peel into fine powder.
- Place powder in a clean glass beaker.
- Add water to it. Keep at room temperature for 24-72 hours.
- Filter it using filter paper
- Collect the filter as banana peel extract.^[10]

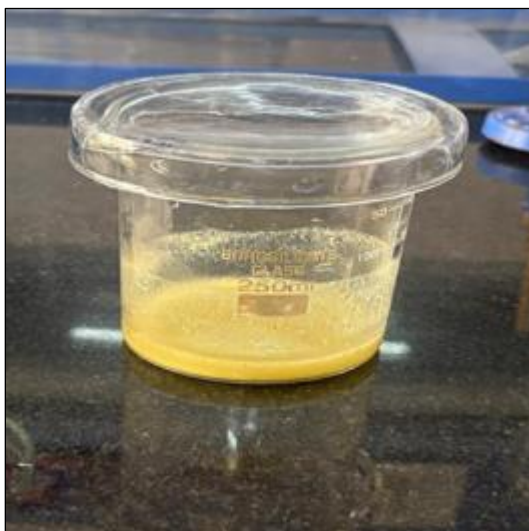


Figure 8: banana peel extract

2.7 METHOD 4: POPPY SEEDS EXTRACT WITH BANANA PEEL EXTRACT

- Cold extract of poppy seeds was prepared and collected in a clean beaker.
- Approximately 1ml of poppy seed extract was measured using a measuring cylinder.
- A small quantity of Banana peel extract was added to the extract.
- The mixture was stirred gently using rod to ensure proper mixing.
- The prepared solution was observed for movement and behavioral act. ^[11]

2.8 EDDY HOT PLATE METHOD ON WISTAR RAT



Figure 9: Wistar Rat

Group	treatment
Control Group	Normal
Standard group	Dioclofenac
Test Group 1	Matricaria chamomilla extract
Test Group 2	Papaver somniferum extract
Test Group 3	Papaver somniferum extract+ Banana peel extract

2.8.1 Procedure

- Animals were randomly allocated to control and test groups based on the study design and housed for acclimatisation under standard laboratory conditions.
- The hot plate apparatus is kept at a controlled, constant temperature as described in the protocol.
- Each animal is placed individually on the heated surface and the baseline pain response (reaction time) is recorded prior to drug administration.
- The experimental design determines the administration of the test drug.
- After a predetermined time interval following the administration, the animal is again placed on the hot plate and the reaction time is recorded.
- Signs of pain sensitivity include licking of the paw, jumping or withdrawal.
- The cut-off time is strictly observed in order to prevent injury to tissue.. 12
- Analgesic activity is measured by comparing control and treated groups.^[12]

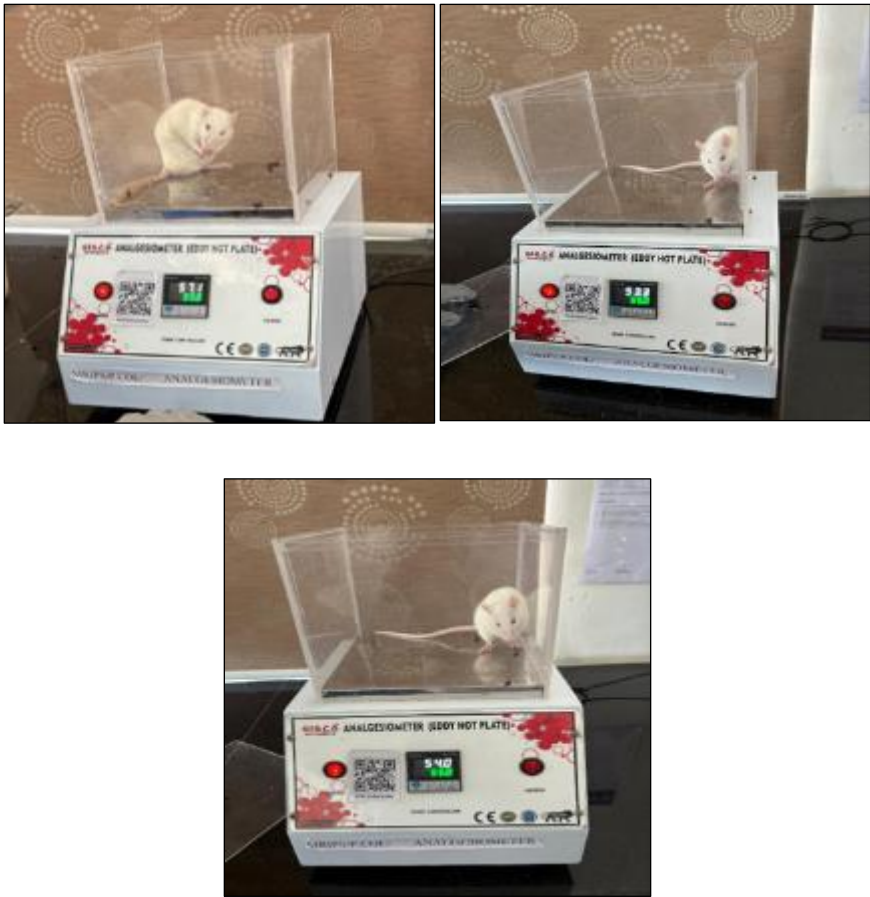


Figure 10: Eddy Hot Plate Method

Table 1: Observation Table

GROUP	TREATMENT	REACTION BEFORE TREATMENT	TIME	REACTION AFTER TREATMENT	TIME	OBSERVATION
CONTROL	Normal Saline	3.2 ± 0.4		3.5 ± 0.5		No Analgesic activity
Standard	Dioclofinac	3.1 ± 0.3		8.9± 0.5		Strong Analgesic activity
TEST 1	Matricacria chamomile extract	3.3 ± 0.5		5.8 ± 0.4		Moderate Analgesic activity
TEST 2	Papaver Somniferumn extract	3.0 ± 0.4		6.9 ± 0.5		Increases in reaction time analgesic activity
TEST 3	Papaver somniferum+Banana peel extract	3.2± 0.3		7.8± 0.4		Higher Analgesic activity

3 RESULT

Table 2: Reaction Time After Treatment

GROUP	MEAN $\pm$ SD
CONTROL	3.5 $\pm$ 0.5
STANDARD	8.9 $\pm$ 0.5
TEST 1	5.8 $\pm$ 0.4
TEST 2	6.9 $\pm$ 0.5
TEST 3	7.8 $\pm$ 0.4

Table 3: Most Animal Study: n=6

Source of Variation	ss	df	MS	F	P- VALUE
BETWEEN GROUPS	99.46	4	24.865	117.85	<0.0001
WITHIN GROUPS	5.28	25	0.211		
TOTAL	104.74	29			

Table 4: Post-HOC Test

GROUP VS CONTROL	MEAN DIFF	95% CI	SIGNIFICANCE
STANDARD VS CONTROL	$\pm$ 5.4	4.68 to 6.12	P<0.001
TEST 3 VS CONTROL	$\pm$ 4.3	3.58 to 5.02	P<0.001
TEST 2 VS CONTROL	$\pm$ 3.4	2.68 to 4.12	P<0.001
TEST 1 VS CONTROL	$\pm$ 2.3	1.58 to 3.02	P<0.001

3.1 High significant

Standard drug show significant analgesic activity < control

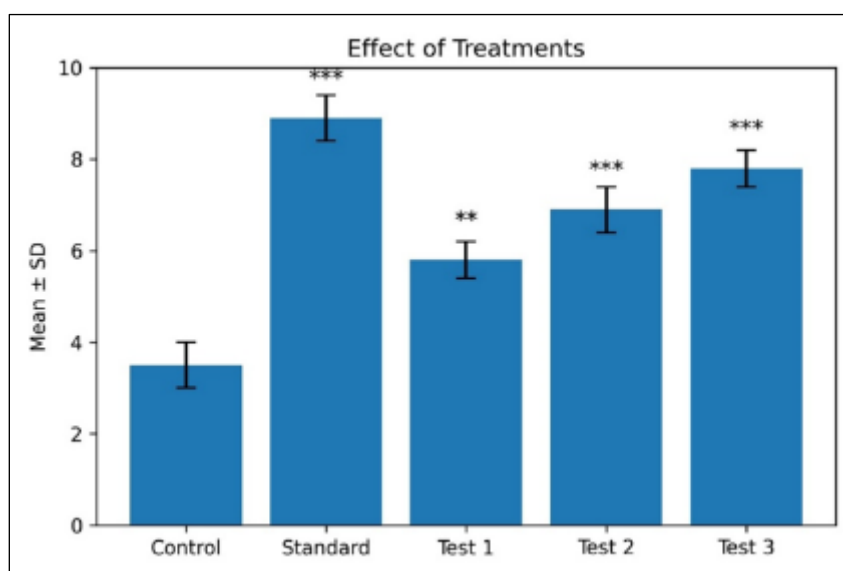


Figure 11: Comparison of Mean Reaction Time Following Different Treatments

4 DISCUSSION

- The control group's reaction time was essentially unchanged.
- Standard drug showed maximum analgesic activity.

Chamomile extract showed mild to moderate analgesic activity. Poppy seeds extract was more effective than chamomile extract for its analgesic effect. The combined presence of poppy seeds extract with banana peel extract showed an enhanced analgesic activity indicating a possible synergic effect. ^[13]

5 CONCLUSION

The study by using Eddy's Hot Plate Method indicated that both poppy seeds extract and chamomile extract possess analgesic properties. The combination of poppy seeds extract and banana peel extract showed higher analgesic effect as compared to individual extract but less than standard drug among the test samples. The increased reaction time observed is suggestive of elevation of pain threshold due to central analgesic activity. Therefore, the combination extract can be a potential natural analgesic agent and needs further pharmacological investigation.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of Conflict of Interest

The authors declare no conflict of interest regarding this manuscript.

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