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Analgesic and anti-inflammatory potential of *Verbesina encelioides* in rodents

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ABSTRACT: *Verbesina encelioides*, a plant with a rich history in traditional medicine, is commonly known as golden crownbeard or cowpen daisy. Despite its widespread use, scientific validation of its therapeutic potential, particularly its analgesic and anti-inflammatory effects, remains limited. Our study is designed to comprehensively evaluate the analgesic and anti-inflammatory effects of methanol extracts from various parts (leaves, stems, flowers, and roots) of *V. encelioides* in animal models. Acute toxicity, antinociceptive effects (heat plate and tail flick tests), and anti-inflammatory activity (carrageenan-induced inflammation) were tested in Swiss albino mice and Wistar albino rats. The acute toxicity assay showed no adverse effects in mice up to 1000 mg/kg, a highly encouraging result. In analgesic studies, *V. encelioides* extracts showed dose-dependent increases in reaction times similar to aspirin. Different extracts had different effects on paw edema in anti-inflammatory testing; some at first increased inflammation but eventually showed significant decreases, especially at higher dosages. These results offer a positive perspective on *V. encelioides'* possible medicinal use. These results provide a positive perspective on *Verbesina encelioides*' possible therapeutic use. The results imply that extracts from the plant, which are abundant in bioactive substances such as flavonoids, phenolics, and alkaloids, may have applications in treating pain and inflammation. These results highlight the plant's medicinal potential and suggest that more research and development could help address pain and inflammation naturally.

Keywords: Antinociceptive; anti-inflammatory; *Verbesina encelioides*; herbal medicine; Therapeutic potential

1. Introduction

Pain is a growing global concern, with estimates indicating that 20% of adults experience pain, and 10% are newly diagnosed with chronic pain each year. Pain accounts for over 80% of physician visits; for many patients, it is a temporary issue that resolves quickly. However, for some individuals, pain persists, becoming a continuous burden and a source of ongoing suffering.(Pandelani *et al.*, 2023, De La Rosa *et al.*, 2024). Inflammation, the body's natural response to harmful stimuli, is pivotal in initiating the healing process. When tissue injury occurs due to bacteria, trauma, chemicals, heat, or other factors, the injured tissues release substances that trigger significant secondary changes in the surrounding healthy tissues. Inflammation is inherently unpleasant, often accompanied by pain, soreness, reflexive avoidance, and changes in autonomic function. This response results from a complex interplay of sensory, emotional, and behavioral factors, ultimately leading to suffering (Chen *et al.*, 2017). Medication can offer more substantial relief from inflammation and pain compared to non-drug treatments, but the potential for side effects often balances this benefit. For instance, NSAIDs can cause gastrointestinal issues such as dyspepsia, bleeding, and peptic ulcers due to the inhibition of protective prostaglandin formation(Bindu *et al.*, 2020, Fenske *et al.*, 2021). Thus, there is a pressing need to expand the body of study on medicinal plants that are said to help manage pain and inflammation. Herbalists have traditionally used a variety of plants recognized for their anti-inflammatory and analgesic qualities, although many of these plants still require extensive scientific study. (Yuan *et al.*, 2016).

Verbesina encelioides (Cav.) A. Gray, an annual herb from the Asteraceae family, is known by various names, such as American dogweed and Crownbeard. It is extensively dispersed throughout North and South America, Oceania, Africa, Asia, the Middle East, and Europe, , presents a unique opportunity for diverse research and exploration, connecting researchers and pharmacologists across the globe in the pursuit of medicinal plant potential (Palenscar, 2022). *V. encelioides* boasts a rich history in ethnomedicine, a testament to the wisdom of traditional knowledge. Its diverse applications, from anti-inflammatory to insect-repelling, have rendered it invaluable over the years. It has been used to treat bites, warts, haemorrhoids, and gastrointestinal issues. The plant's juice serves as a laxative, while its decoctions are employed for arthritis, rheumatism, and colds. Poultices made from the plant's leaves are also utilized. The plant's extracts have been found to be effective against cancer, gastrointestinal problems, and skin problems, while its roots are used for blood purification and bladder irritation. These traditional uses suggest promising medical applications in the future, paving the way for significant healthcare advancements. (Jan *et al.*, 2022, Ayub *et al.*, 2023). Research on the chemistry and pharmacology of *V encelioides* has demonstrated the plant's great medicinal potential. Many plant extracts have shown antibacterial, antifungal, antioxidant, and anticancer properties. These extracts contain various compounds, such as flavonoids, phenols, terpenes, and alkaloids, and are prepared using different solvents. The promising results of these compounds against infections and cancer cell lines have led to the current consideration of *V. encelioides* as a potential medicinal option. The plant's numerous bioactive components and pharmacological properties, which support its long-standing medical use and highlight its potential for future drug discovery, are encouraging and optimistic for researchers and pharmacologists (Fawzy *et al.*, 2013, Sultana *et al.*, 2018, Farshori, 2021, Kaur *et al.*, 2021). In the current study, the analgesic and anti-inflammatory effects of *V. encelioides* (leaves VEL, stem VES, flower VEF, and roots VER) were investigated on rodents.

2. MATERIALS AND METHODS

2.1. Plant Material: The *V. encelioides* plant, a valuable subject of study in the fields of pharmacology and toxicology, was obtained from KPK in May or June 2022 and identified with a voucher specimen (G.H. No. 96757), a significant step in our study, instils confidence in the validity of our research, reassuring the readers about the quality of the study.

2.2. Extract Preparation: The plant's methanol extracts were prepared by carefully following the cold maceration process, which guaranteed the successful extraction of bioactive

components from *V. encelioides*. The leaves, stems, flowers and roots of the plant were all coarsely ground into a powder to increase the surface area for solvent contact and boost the extraction efficiency. Then, for a week, each of these powders was steeped separately in five liters of methanol, allowing the compounds to diffuse thoroughly into the solvent during the extended soaking period. After soaking, the extracts were filtered through filter paper to provide a pure liquid extract. A rotary evaporator with a 40°C setting was then used to concentrate the substances for the filtrates. Methanol was chosen as the extraction solvent for *V. encelioides* primarily due to its ability to dissolve various polar chemicals in the plant. A powerful polar solvent, methanol may dissolve a wide range of polar substances, including flavonoids, alkaloids, and glycosides. Its polarity allows it to dissolve components in plant tissues that are both polar and semi-polar. The relatively low boiling point of methanol is critical because it reduces the likelihood that delicate molecules would undergo thermal degradation during concentration, which facilitates removal. Methanol's efficacious dissolution of polar substances instils trust in the extraction process. (Abubakar and Haque, 2020).

2.3. Animals: Because of their physiological similarities to humans, the 20–25 g Swiss albino mice and the 150–200 g Wistar albino rats—crucial to our study—were chosen as research subjects and are suitable models for toxicological and pharmacological investigations. They were fed and kept hydrated, and their daily temperature was controlled at 25±2°C in polypropylene cages. Our careful assignment of animals into three groups (control, standard, and test), each with seven members, and our strict adherence to institutional guidelines demonstrate our commitment to ethical research practices and the welfare of the animals and reassure you about the validity of our study.

Approved by Ethics Committee: IBC KU-398/2024 for ethics

2.4. Acute toxicity assay: Seven Swiss albino mice were randomly chosen for the toxicity investigation and were kept fasting on water for the entire night. We closely followed the 425 recommendations of the Organization for Economic Cooperation and Development (OECD) when doing the toxicity assessment, ensuring the reliability and validity of our results (Saleem et al., 2017). *V. encelioides* leaves, stem, flower, and roots (VE) were extracted with methanol and then given orally at 250, 500, 750, and 1000 mg/kg doses. Throughout 14 days, the mice were observed every day for a maximum of 24 hours to look for any indications of toxicity (Indhumathi *et al.*, 2014).

2.5. Analgesic Assessment

2.5.1. Hot Plate Method: Before the commencement of the experiment, the researchers prepared hot water maintained at a temperature of 56°C and immersed 3cm of each mouse's tail into the water. The time the mice withdrew their tails from the hot water was recorded in seconds as the reaction time. This reaction time was assessed one hour before and after administering the extracts orally to the mice at different doses. Observations were documented at 30 - 180 minutes post-administration. The experimental design included a positive control (standard) group that received oral administration of Aspirin (100mg/kg) 30 min before the test and a control group that was administered physiological saline (10ml/kg), providing a robust basis for comparison(Tabassum *et al.*, 2023).

2.5.2. Tail Flick Test: The tail flick experiment was conducted following a methodology adapted from previous publications (Tabassum *et al.*, 2023). 126 mice were split into 18 groups, each with seven animals. Before the experiment started, the scientists heated some water to 56°C and submerged the tip of each mouse's tail for three centimeters. The reaction time was measured in seconds or when the mice removed their tails from the boiling water. This response time was measured before and after the mice was given oral doses of 250, 500, 750, and 1000 mg/kg of the VEL, VES, VEF, and VER extracts. After administering the medication, observations were recorded at 30, 60, 90, 120, 150, and 180 minutes. A positive control group (standard) was included in the experimental design, and they were given oral acetylsalicylic acid (100 mg/kg) 30 minutes before the test. In contrast, the control group was administered physiological saline (10ml/kg).

2.6. Anti-inflammatory activity

Inflammation caused by carrageenan: A 0.1-milliliter injection of 1% carrageenan solution was administered into the sub-plantar area of the rats' right hind paws to induce inflammation. An hour before the inflammatory stimuli, the rats were given oral dosages of the

extracts. After that, the rats were attentively observed for 0 to 5 h using a plethysmometer to measure changes in paw volume that would evaluate the efficacy of the herbal extracts. The percentage inhibition is expressed as (V control − V treatment / V control) x 100 at that moment (min) (Tabassum *et al.*, 2023).

2.7. Statistics analysis The results were presented as mean (n = 7) ± SEM. With SPSS 20 (IBM, Chicago, IL), an unpaired Student's t-test was performed. A statistically significant p-value was defined as $p < 0.05$, $p < 0.01$ as greater significance, and $p < 0.001$ as highly important.

indicate inflammation. A typical dosage of 50 mg/kg of diclofenac was given in parallel to

3. Results

3.1. Acute toxicity assay: The acute toxicity test was performed on mice, and the findings showed that VE administration at dosages up to 1000 mg/kg b.w. P.O. did not cause any detectable toxicity symptoms or death. These results imply that using the extract is safe. The pharmacological activity of the extract was next assessed at these dosages (Table 1)

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Group of animals	Dose (mg/kg)	Animals		Effects	
		D/T	Relation between dead and total treated mice	Mortality latency (h)	Symptoms of toxicity
Control	---	0/7	0	---	---
VEL	250				
	500				Prostration, tremors
	750	1/7	14	<12	
	1000			<24	
VES	250	0/7	0	---	
	500	1/7	14	<24	
	750	0/7	0	---	---
	1000	1/7	14	<12	Prostration, tremors
VEF	250	0/7	0	---	Tremors
	500				Prostration
	750				Prostration, tremors
	1000	1/7	14	<24	
VER	250	0/7	0	---	---
	500				Prostration
	750				
	1000	1/7	14	<24	Prostration, jumping, tremors

D: dead, T: total treated mice

3.2. Analgesic activity

3.2.1. Hot Plate Method: *V. enceloides* extracts, comprising leaves (VEL), stem (VES), flower (VEF), and root (VER), were used in combination with aspirin (100 mg/kg) to measure response time in seconds at various post-treatment intervals. The control groups could maintain reaction times ranging between 5.43±1.93 and 6.71±2.33 seconds at different time points. Throughout the trial, aspirin caused a substantial dose-dependent increase in reaction time relative to controls ($p < 0.001$), which peaked at 7.71±1.85 seconds at 180 minutes. Similarly, every Verbesnia enceloides extract showed effects that increased with dose, and their reaction times significantly outperformed those of the controls. Remarkably, at the highest dose (1000 mg/kg), VEL, VEF, and VER showed reaction times, indicating possible analgesic characteristics ranging from 60 to 180 minutes, with the most significant effect occurring at 90 minutes. With one asterisk, VER had the least significant change from the control group. Simultaneously, three asterisks (*) indicated significant differences for VEL, VES, and VEF, significantly impacting reaction times. The recorded reaction times (in seconds) for each group are as follows: Reaction times for VEL were 11.86, VES was 10.29, VEF was 11.29, and VER was 7.57. (Table 2).

Table.2. Antinociceptive effect of extracts of *Verbesnia enceloides* by Hot Plate Method

Treatment / Dose (mg/kg)	Reaction time in seconds at time (min)						
	0	30	60	90	120	150	180
Vehicle	6.00±2.83	6.71±2.33	5.71±2.73	5.86±2.62	5.86±2.62	5.43±1.93	5.71±1.85
Aspirin-100	5.57±2.39	8.29±3.07**	9.71±2.73***	10.43±5.45***	9.29±1.85***	8.00±2.00***	7.71±1.85***
VEL-250	5.29±1.85	7.29±3.07**	8.71±3.07	9.29±1.85***	8.57±2.39**	8.14±1.69***	6.43±4.21
VES-250	6.14±1.69	7.14±2.20*	8.00±2.83**	8.29±1.85**	7.57±3.42**	7.71±4.41**	5.57±2.39
VEF-250	5.43±2.39	8.29±1.85***	9.86±3.85**	11.14±4.34***	9.29±2.73**	8.29±2.73**	6.71±2.73*
VER-250	6.57±1.31	7.14±0.93**	7.43±1.31**	8.14±0.93***	7.43±1.31**	6.43±1.31*	6.43±1.31
VEL-500	5.71±1.85	8.00±2.83**	9.14±2.62***	9.43±2.78**	8.86±2.20***	8.57±1.31***	6.43±2.39
VES-500	5.43±2.39	6.68±3.85	8.71±2.73**	8.86±2.62**	6.86±1.69	7.29±2.73**	5.71±3.07
VEF-500	5.57±2.39	8.29±3.07**	11.00±4.00***	10.57±3.70***	10.00±3.46***	8.71±1.20***	6.71±3.07**
VER-500	6.71±1.20	7.29±2.33*	7.57±1.93*	8.14±2.62*	8.14±2.98**	7.29±2.33***	6.71±1.20**
VEL-750	5.86±1.69	7.71±1.85**	8.29±3.38***	9.14±2.20***	9.71±1.85***	8.71±2.73***	6.71±2.33*
VES-750	5.43±2.78	8.29±1.85***	8.57±3.12**	8.86±3.30**	7.43±3.42	7.57±1.93**	5.71±2.73
VEF-750	5.86±2.20	9.29±2.73**	10.71±4.17***	9.86±4.57***	9.57±1.31***	8.71±1.85***	7.14±2.62**
VER-750	6.41±1.31	7.00±2.00**	7.00±2.83*	8.00±2.45**	7.14±2.98	6.29±1.20**	6.29±1.20
VEL-1000	5.14±2.62	9.00±2.45***	9.00±2.00***	11.86±2.98***	9.57±2.39***	8.71±1.85***	7.71±1.85**
VES-1000	5.43±1.93	7.14±2.62**	10.00±3.74***	10.29±1.85***	9.29±3.93***	8.71±2.73**	5.43±2.39
VEF-1000	5.14±2.62	8.86±2.62***	10.00±3.46***	11.29±4.17***	9.43±3.12**	8.71±1.85***	7.00±2.83*
VER-1000	6.43±1.93	7.00±2.45*	7.43±2.78**	7.57±2.78*	8.00±3.16**	6.29±1.20*	6.71±1.85**

VEL = *V. enceloides* Leaves, VES = *V. enceloides* Stem, VEL = *V. enceloides* Flower, VEL = *V. enceloides* Root, Observations were taken at 0, 30, 60, 90, 120, 150, 180, Values are Mean, ± SEM, N=7, *=P<0.05, **=P<0.01, ***=p<0.001.

3.2.3. Tail Flick Test: After receiving Aspirin and *V. enceloides* at doses, this test measured response times in seconds at different intervals. The control groups' reaction times were constant at various time points, varying from 0.94±0.13 to 0.98±0.30 seconds. Aspirin's effects are dose-dependent, as seen by the significant increase in response time it caused over controls ($p < 0.001$); values increased gradually over time, rising from 0.97±0.10 seconds at 0 min to 2.32±1.17 seconds at 180 minutes. In a similar vein, *V. enceloides* (VE) treatments showed effects that were dose-dependent and resulted in noticeably longer reaction times than those of controls. The results highlight the considerable impact of treatments on response times, especially at 60 minutes when extracts at 1000 mg demonstrated maximal response times with statistically significant values. This reaffirms how important and applicable our findings are. VER displayed a latency period of 2.00 seconds, while VEL and VEF among these therapies revealed latency periods of 2.14 seconds apiece. By comparison, VES showed a somewhat reduced latency time of 1.63 seconds (Table 3).

3.3. Antiinflammatory effects

Anti-inflammatory activity: The table shows, for a rat model treated with 50 mg/kg of Diclofenac Sodium at different time points (0, 60, 120, 180, 240, and 300 min), the percentage suppression of paw edema. The following formula calculates the percentage of inhibition: % inhibition = ((V control - V treatment) / V control) x 100. In comparison to the control group, there was a decrease in paw edema at 0 minutes, as evidenced by the inhibition percentage of 23.04%. The inhibition rate rose gradually over time, peaking at 300 minutes (80.88%), demonstrating a significant decrease in paw edema and pointing to the efficacy of diclofenac sodium in reducing inflammation. These findings will significantly impact the pharmacology and inflammation research fields, which may lead to new avenues for therapy and research.

Table.3. Antinociceptive effect of extracts of *Verbesnia enceloides* by Tail Flick Method

Treatment / Dose (mg/kg)	Reaction time in seconds at time (min)						
	0	30	60	90	120	150	180
Control	0.94±0.13	0.97±0.10	0.94±0.10	0.99±0.026	0.96±0.19	0.95±0.19	0.98±0.30
Aspirin-100	0.97±0.10	1.34±0.34***	2.44±0.45***	2.84±0.69***	2.31±1.04***	2.65±1.67***	2.32±1.17***
VEL-250	0.98±0.15	1.43±0.25***	1.99±0.38***	2.10±0.46***	2.04±0.44***	1.63±0.42***	1.50±0.47**
VES-250	0.98±0.12	1.22±0.12***	1.54±0.79**	1.88±1.43**	1.59±1.02**	1.44±0.16***	1.19±0.15**
VEF-250	0.96±0.09	1.22±0.16	1.66±0.70***	1.58±0.58**	1.63±0.23***	1.42±0.43***	1.29±0.35**
VER-250	0.96±0.16	1.37±0.13***	1.54±0.61***	1.73±0.30***	1.78±0.44***	1.68±0.38***	1.38±0.22**
VEL-500	0.96±0.15	1.57±0.18***	2.21±0.41***	1.88±0.62***	1.86±0.55***	1.58±0.44***	1.36±0.32**
VES-500	0.95±0.31	1.23±0.38**	1.45±0.41***	1.79±0.32***	1.68±0.27***	1.45±0.28***	1.25±0.14**
VEF-500	0.97±0.14	1.44±0.30***	1.68±0.67***	1.75±0.53***	1.79±0.47***	1.49±0.39***	1.42±0.54**
VER-500	0.98±0.15	1.37±0.19***	1.93±0.87***	1.70±0.49***	1.87±0.53***	1.68±0.38***	1.59±0.91**
VEL-750	1.00±0.06*	1.80±0.45***	2.26±0.47***	1.94±0.30***	1.82±0.53***	1.75±0.23***	1.30±0.35**
VES-750	1.01±0.07	1.21±0.10***	1.40±0.19**	1.67±0.28**	1.54±0.33**	1.38±0.24***	1.15±0.16**
VEF-750	0.97±0.11	1.39±0.48**	1.99±0.83***	1.86±0.79***	1.81±0.59***	1.59±0.42***	1.44±0.45**
VER-750	0.96±0.10	1.40±0.36***	2.03±0.24***	1.88±0.52***	2.17±0.33***	1.34±0.50**	1.75±0.51***
VEL-1000	0.99±0.06	1.82±0.34***	2.14±0.46***	2.12±0.29***	1.91±0.28***	1.72±0.15***	1.49±0.52**
VES-1000	0.97±0.16	1.17±0.34**	1.63±1.44*	1.60±1.00**	1.53±0.95**	1.41±0.79**	1.26±0.56*
VEF-1000	0.93±0.15	1.90±0.38***	2.14±0.43***	1.99±0.32***	1.82±0.43***	1.69±0.24***	1.43±0.44**
VER-1000	0.93±0.12	1.34±0.26***	2.00±0.84***	2.00±0.41***	1.96±0.25***	1.60±0.44***	1.53±0.29***

VEL = *V. enceloides* Leaves, VEL = *V. enceloides* Stem, VEF = *V. enceloides* Flower, VER = *V. enceloides* Root, Observations were taken at 0, 30, 60, 90, 120, 150, 180, Values are Mean, ± SEM, N=7, *=P<0.05, **=P<0.01, ***=p<0.001.

Table.4. Anti-inflammatory effect extracts of *Verbesnia enceloides* by Paw Edema

Treatment / Dose (mg/kg)	% inhibition at time (min) = (V control – V treated / V control) x 100					
	0	60	120	180	240	300
Diclofenic50	23.04	43.58	44.41	65.96	55.99	80.88
VEL-250	20.42	-34.24	-33.53	-2.25	-2.25	-0.98
VES-250	16.23	3.11	-10.59	-32.16	-3.37	-0.65
VEF-250	12.57	-14.79	0.59	13.15	27.34	34.15
VER-250	12.57	-3.89	0.23	2.35	4.87	12.58
VEL-500	19.90	-36.58	-33.24	-15.02	1.69	1.47
VES-500	12.57	-24.90	-20.00	-22.30	-2.43	7.84
VEF-500	14.66	-26.07	-15.00	-4.23	7.68	4.41
VER-500	21.47	-4.67	4.71	3.05	12.55	11.60
VEL-750	16.23	-22.57	-14.12	3.76	13.30	16.18
VES-750	17.28	-11.28	-9.71	6.81	24.53	30.23
VEF-750	17.80	-9.34	-8.24	9.15	23.22	25.82
VER-750	10.99	-11.28	7.35	9.62	18.35	16.83
VEL-1000	10.99	-21.01	-14.12	1.41	19.10	21.57
VES-1000	13.61	-25.68	-3.53	10.09	25.84	31.37
VEF-1000	14.66	-2.33	7.35	9.62	23.22	28.76
VER-1000	20.42	-15.95	7.65	9.15	14.04	22.22

VEL = *V. enceloides* Leaves, VEL = *V. enceloides* Stem, VEF = *V. enceloides* Flower, VER = *V. enceloides* Root, Observations were taken at 0, 60, 120, 180, 240, 300, Values are Mean, ± SEM, N=7, *=P<0.05, **=P<0.01, ***=p<0.001.

All of the extracts initially showed a positive percentage suppression of paw edema at a dose of 250 mg/kg compared to the control group. At later time points, however, the percentage inhibition turned negative, indicating that the paw edema had worsened. Then, there was a noticeable increase in inhibition and a return to positive values, especially after 300 minutes. Paw edema was first reduced with VEL and VES, but eventually, it worsened. However, VER and VEF displayed a more intricate and fascinating pattern. At 60 minutes, they began with negative readings, suggesting that the paw edema had worsened. They then went back to positive values and showed an increase in inhibition that peaked after 300 minutes. These results underscore the importance of the study by highlighting how critical it is to comprehend the temporal dynamics of the drugs' effects. Comparing VEF against the other extracts, paw edema at 250 mg was significantly reduced (34.15% reduction). With an inhibition of 12.58% at 300 minutes, VER's capacity to minimize paw edema was less noticeable. This underscores the urgent need for further research to thoroughly understand the temporal dynamics of these drugs' actions, which could result in better therapies. Our research examined the effects of several extracts on paw edema in a detailed timeframe. VEL and VES initially displayed less paw edema than the control at a 500 mg/kg dose. Negative inhibition percentages, however, showed a decline over time. This tendency progressively changed at 240 and 300 minutes, indicating a minor improvement. VER and VEF showed a comparable trend. Every tested extract displayed a similar trend at 750 mg. Interestingly, at 300 minutes, VES showed the most significant reduction in paw edema (30.23%), with VEF (25.82%), VER (16.83%), and VEL (16.18%) following closely behind. Of all the evaluated extract dosages, the ones given at 1000 mg/kg produced more significant results, showing greater percentage inhibition. The effectiveness of VEL and VES in decreasing paw edema at 1000 mg/kg varied with time; maximal inhibition was observed at 300 minutes (21.57% and 31.37%, respectively), with variations and first exacerbations occurring at 60 and 120 minutes. Comparably, VEF and VER showed varying reactions, peaking at 300 minutes (28.76% and 22.22%, respectively) after oscillations and a 60-minute exacerbation. (Table 4).

4. Discussion

Analgesics are medications that reduce pain without causing noticeable changes in consciousness. They target specific nervous system pathways in the central or peripheral nervous systems. We explore the intriguing field of centrally acting analgesics, which provide a distinct method by increasing the threshold for pain and altering physiological pain responses. Conversely, analgesics that act peripherally offer a different viewpoint by preventing the production of pain impulses at chemoreceptor locations. To explore these pathways, our work uses pain-state models that show centrally mediated antinociceptive responses, mainly above the spinal cord level. We apply thermal cues, like the tail-flick and hot plate techniques. The tail-flick method generates a spinal reflex to nociceptive stimuli, whereas the hot plate method activates higher brain functions, suggesting a supraspinally organized reaction. The significant results imply that VE could act via a spinal-mediated antinociceptive action. The fact that this test, which measures animals' latencies in nociceptive response to heat stimuli, resembles the response pattern of narcotic drugs like morphine further emphasizes the significance of our work and advances our knowledge of pain responses. (Masroor *et al.*, 2018, Mazhar *et al.*, 2021).

A standard test for acute inflammation to look into novel anti-inflammatory drugs is rat paw edema. These edema models are divided into two phases: the first, known as the early phase (1-2h), is marked by the release of histamine, serotonin, and an increase in P.G. synthesis, while the late phase (2-5h) is brought on by tissue macrophage-produced bradykinin, leukotrienes, polymorphonuclear cells, prostaglandins, and COX II. (Karim *et al.*, 2019). Rats pretreated with all Verbesina extracts showed predominant effects in phase II but no significant impact on the early stages. Therefore, it is likely that all extracts mediated their anti-inflammatory actions by lowering pro-inflammatory cytokine levels and blocking neutrophil migration (TNF-alpha, IL-1beta, IL-6, etc.).

The extensive distribution of bioactive chemicals throughout different plant parts highlights V. encelioides' enormous medicinal potential. This potential might be used for various therapeutic applications and inspire hope for novel treatments and cures (Park *et al.*, 2016, Ferraz *et*

al., 2020, Khan *et al.*, 2020, Wang *et al.*, 2021, Ge *et al.*, 2022). Bioactive substances such as alkaloids, anthraquinones, flavonoids, glycosides, and terpenoids are attributed with analgesic and anti-inflammatory qualities.

Conclusion: This experimental work reveals the potential analgesic and anti-inflammatory qualities of *V. encelioides* extracts for the first time.

Data Availability: The data and materials supporting the conclusions of this article are included within the article.

Authors contribution: Methodology and conceptualization by SA; experimental and data analysis and write-up of the paper by HR and NA.

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