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Research Article

An Experimental Study Comparison of the Analgesic and Antioxidant Effects of Morphine and 7-Hydroxyflavone in Rats

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Abstract

Background: Plants have played a crucial role in human health since ancient times, serving as reliable and significant sources of biologically active compounds used to treat numerous disorders. Thus, the potential of these compounds to treat various diseases, including cancer and cardiovascular conditions, is being extensively researched. Notably, the diverse mechanisms of action associated with these compounds make them promising candidates for future drug development. Meanwhile, 7-hydroxyflavone (7-HF), a natural flavonoid, has demonstrated notable biological properties; however, the therapeutic potential of 7-HF for pain remains unexplored. Threfore, this study aimed to investigate the potential role of 7-HF in alleviating pain in rats. **Materials and Methods**: This prospective experimental study employed healthy Swiss albino rats with Institutional Animal Ethical Committee (IAEC) approval. Rats were divided in to four groups: control group, which received normal saline; the standard group, which received morphine 2 mg/kg; and two test group, which received 7-HF at doses of 5 & 10 mg/kg/rat. Behavioral analysis was performed after treatment to assess the central and peripheral analgesic effects using the hot plate, tail flick analgesiometer, tail immersion, and acetic acid-induced writhing, a validated animal model for screening analgesic effects in a preclinical laboratory. All animals were sacrificed after the writhing test and screened for oxidative stress markers as well as antioxidants level. **Results**: At 30–240 min, the administration of a 10 mg/kg test dose notably showed prolonged response time in the hot plate test, tail flick test and tail immersion method. In the acetic acid writhing test, 7-HF at 10 mg/kg significantly inhibited writhing in experimental animals. Moreover, 7-HF at a dose of 10 mg/kg restored superoxide dismutase (SOD) and glutathione (GSH) levels, and conversely reduced elevated lipid peroxidation (LPO) levels. **Conclusion**: This study provides the first evidence that 7-HF effectively mitigates pain in rats, likely through the associated antioxidant, anti-inflammatory, and analgesic effects. These results support the therapeutic potential of 7-HF as a novel candidate for a management pain in various chronic disorders.

Keywords: analgesics; 7-hydroxy flavone; oxidative stress; acetic acid induced writhing; hot plate; pain measurement

1. Introduction

Pain is a complex protective response that arises when tissue injury or dysfunction disrupts physiological homeostasis. It is transmitted through ascending nociceptive pathways involving the spinothalamic tract, thalamus, midbrain, and pons before reaching the somatosensory cortex, where the intensity, location, and severity of pain are perceived. Although pain serves as an essential defense mechanism, persistent or pathological pain significantly impairs quality of life and represents a major global clinical burden [1–3].

Despite advances in pharmacological management, effective treatment of pain remains a major clinical challenge. Opioid analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) are the mainstay of pain therapy; however, their long-term use is frequently associated with serious adverse effects. Opioids are well known to cause tolerance, physical dependence, and addiction [4,5]. Whereas NSAIDs are commonly linked to gastrointestinal irritation, renal dysfunction, and increased cardiovascular risk. Moreover, these conventional analgesics often provide only partial relief, with many patients experiencing inadequate pain control [6–8]. Collectively; these limitations underscore the urgent need to develop safer and more effective non-opioid analgesic strategies for the management of acute and chronic pain.

In earlier studies, natural bioactive compounds have gained increasing attention as potential non-opioid analgesics due to their multi-target mechanisms and favorable safety profiles [8]. Among these, flavonoids represent a large class of polyphenolic compounds widely distributed in fruits and medicinal plants, exhibiting antioxidant, anti-inflammatory, and neuroprotective properties [9,10]. Furthermore, previous research has demonstrated that flavonoids can modulate pain and inflammation by inhibiting cyclooxygenase (COX) and lipoxygenase (LOX)

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enzymes, suppressing oxidative stress, and interacting with key neurotransmitter systems, including γ-aminobutyric acid (GABA), serotonin, dopamine, and glycine [11–13]. Notably, flavonoids such as epigallocatechin-3-gallate (EGCG) have shown significant analgesic and anti-inflammatory effects in both preclinical and clinical studies, supporting their translational potential as non-opioid therapeutic agents [14–16].

7-Hydroxyflavone (7-HF), a naturally occurring flavonoid isolated from *Oxytropis falcata* Bunge, a traditional Tibetan medicinal plant, has been reported to exhibit a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antiplatelet, and organ-protective effects [17–19]. Mechanistic studies have demonstrated that 7-HF activates the Nrf2/NQO1/HO-1 signaling pathway, thereby enhancing endogenous antioxidant defenses, as evidenced by increased superoxide dismutase activity and reduced lipid peroxidation [20]. In addition, 7-HF has been shown to suppress pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) through modulation of the MAPK/NF-κB signaling pathway [21]. Importantly, experimental evidence indicates that 7-HF exerts significant analgesic effects by activating ATP-sensitive potassium channels and by dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) enzymes, resulting in attenuation of inflammatory edema and nociceptive pain [21,22].

Safety evaluations further support the therapeutic potential of 7-HF. Developmental toxicity assessments using zebrafish embryo-larval models have shown no significant adverse effects on growth or behavior, indicating a favorable safety profile [23].

Despite such promising pharmacological activities, no study has systematically evaluated the analgesic efficacy of 7-HF, and its underlying mechanisms have not been comprehensively investigated using both centrally and peripherally mediated pain models. Therefore, the present study was undertaken to investigate the analgesic and antioxidant effects of 7-hydroxyflavone using established experimental pain models and to elucidate its possible mechanisms of action. This investigation aims to contribute to the development of safer, non-opioid therapeutic strategies for effective pain management.

2. Materials and Methods

2.1 Drugs and Chemicals

All drugs used in treatment protocols were obtained from different sources. 7-Hydroxyflavone (7-HF) was purchased from PP Enterprises, Chhatrapati Sambhajinagar, Maharashtra, India. Morphine was obtained from Rajarshi Shahu College of Pharmacy, Buldana. Additional chemicals, including 7-CMC, DMSO (dimethyl sulfoxide), saline, DTNB (5,5'-dithio-bis-2-nitrobenzoic acid), phosphate buffer, hydrogen peroxide, glacial acetic acid, thiobarbituric acid, sodium dodecyl sulfate, distilled water, n-butanol, and pyridine, were of analytical grade.

2.2 Experimental Animal

Healthy Wistar rats with 180–200 g body weight were used for this study. The animals were sourced from the Department of Pharmacology, Rajarshi Shahu College of Pharmacy, Buldhana, Maharashtra, India. The animals were housed in well-ventilated polypropylene cages (six animals per cage) under standard laboratory animal house conditions (12-hour light/12-hour dark cycle, temperature of 25 ± 2 °C, and relative humidity of 45–55%) with free access to food and water throughout the study. The experimental procedure was approved by the Institutional Animal Ethics Committee under registration number IAEC/1865/24-25/P-11 (Registration No. 1865/PO/16/CPCSEA) and conducted in accordance with CPCSEA guidelines (Ministry of Environment, Forests, and Climate Change, Government of India).

2.3 Experimental Design

Experimental Animal Grouping

Twenty-four Wistar rats of either sex were randomly allocated into the following four groups (n = 6) as follows: **Group I** (Normal Control) received vehicle (0.9% NaCl) orally. **Group II** (Standard) received Morphine Sulphate 2 mg/kg i.p.; **Groups III-IV** (Test groups) received 7-Hydroxyflavone (5 & 10 mg/kg in 0.5% CMC orally). The experimental design was depicted in Fig. 1, and the grouping of animals was shown in Table 1 The doses of 7-hydroxyflavone (5 and 10 mg/kg) were selected based on earlier reports demonstrating pharmacological efficacy of flavone derivatives within this dose range. The selected doses allowed evaluation of dose-dependent effects. The individual's animals in each group received the treatment before the experiment, and they were evaluated for the analgesic test after the treatment at 30, 60, 120, and 240 minutes respectively. Animals were monitored daily throughout the entire experimental period for general health, behavior, and signs of distress. Behavioral and biochemical assessments were conducted according to the experimental timeline, and animals were observed until completion of the study.

2.4 Assessment of the Analgesic Effect of 7-Hydroxyflavone Using Animal Model

2.4.1 Hot Plate Method

According to Eddy and Leimbach's detailed method [24], the hot plate is a valid model that is often used in preclinical research to screen the analgesic effect of medicines. Rats were kept on a hot plate with a constant temperature of 55 ± 1 °C for this experiment. Animals' times for jumping or licking their paws were recorded. To determine the rats' response to electrical heat-induced pain, each rat was placed separately on the hot plate (licking of the forepaws

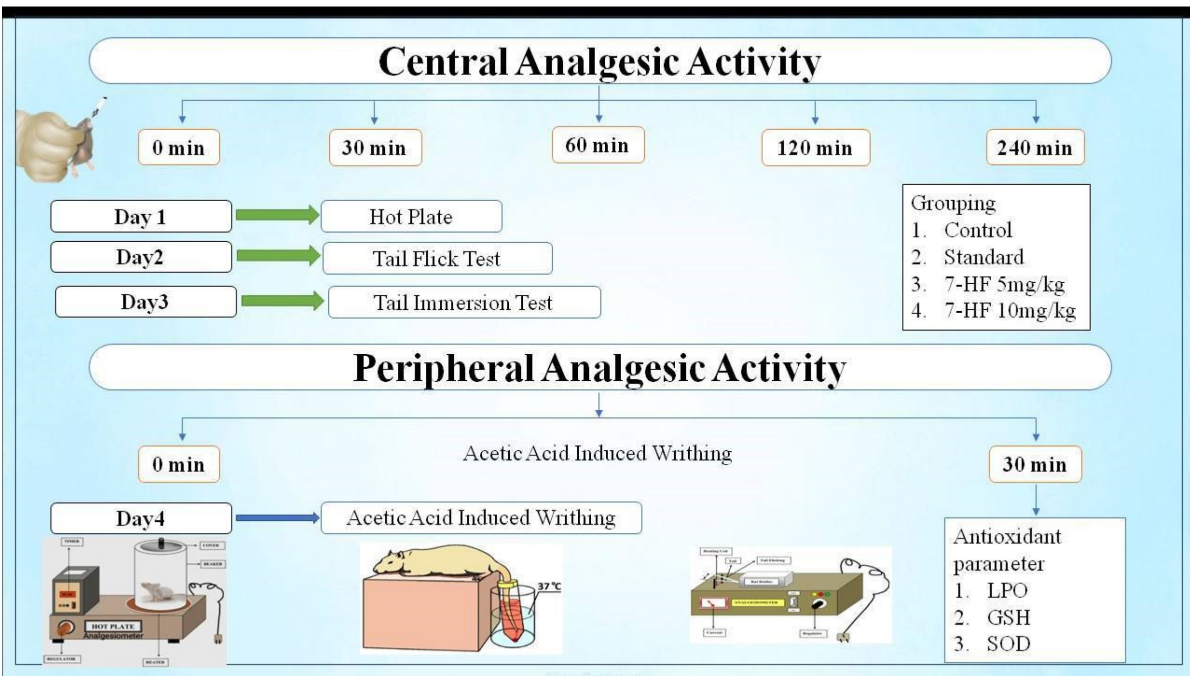

Fig. 1. Experimental design. 7-HF, 7-hydroxyflavone; LPO, lipid peroxidation; GSH, glutathione; SOD; superoxide dismutase.

Table 1. Experimental groups and treatment used for the evaluation of analgesic activity in animals using the hot plate, tail flick, tail immersion, and acetic acid–induced writhing tests.

Sr. no.	Groups	No. of animal	Treatment
1	Normal	6	Normal saline 1 mL/kg (p.o)
2	Standard	6	Morphine Sulphate 2 mg/kg (i.p.)
3	Test1 (low dose)	6	7-Hydroxyflavone 5 mg/kg (p.o)
4	Test2 (high dose)	6	7-Hydroxyflavone 10 mg/kg (p.o)

After the completion of the experimental investigation, rats were anesthetized with ketamine (50 mg/mL, 90 mg/kg body weight, i.p) and xylazine (20 mg/mL, 10 mg/kg, i.p). Blood sample was obtained via retro-orbital puncture.

and eventually jumping). Following the administration of normal saline, 7-hydroxyflavone (5 & 10 mg/kg), and morphine sulfate (2 mg/kg), the rats' response was recorded before and after 30, 60, 120, and 240 minutes. The latency until the rats displayed the first signs of discomfort (hind paw lifting, hind paw licking, or jumping) was recorded [24,25].

2.4.2 Tail Flick Test

The tail flick test was utilized to determine analgesic effects using procedure highlighted by D'amour and Smith (1941) [26]. With little modification to the procedure, the anti-nociceptive effect in rats was evaluated using the tail flick method. The study employed a radiant heat automatic tail flick analgesiometer, which was utilized to assess pain response as tail flick latencies. The animals' basal reaction time to heat was evaluated by placing the tip of the tail 1–2 cm on a radiant heat source. The termination point was determined by removing the tail from the radiant warmth. The 15-second time limit considered as cutoff time was applied to prevent additional heat-related harm or damage to the tail. Four groups (n = 6) of rats were treated. Following the administration of normal saline, 7-hydroxyflavone (5 & 10 mg/kg), and morphine sulfate (2 mg/kg), the tail-flick response time was evaluated before and after 30, 60, 120, and 240 minutes after treatment [25,27].

2.4.3 Tail Immersion Method

An assessment of 7-hydroxyflavone's analgesic effect was conducted using a tail immersion test on rats, following the protocol proposed by Aydin *et al*. (1999) [28]. The rats' lower 5 cm of tails were immersed in a beaker with water kept at a constant temperature of 55 $\pm$ 0.50 °C. The reaction time was recorded as the time it took for the tail to be withdrawn out of the water in seconds, with a 15-second immersion cutoff time. The reaction time was evaluated before and after 30 minutes, 60 minutes, 120 minutes, and 240 minutes. Following the administration of normal saline, 7-hydroxyflavone (5 & 10 mg/kg), and morphine sulfate (2 mg/kg) [28].

2.4.4 Acetic Acid-Induced Writhing Test

The test for writhing in response to acetic acid was conducted using the methodology outlined by Sharma *et al.*, 2019 [29]. In this paradigm, rats were treated with morphine sulfate 2 mg/kg, a standard drug, and 7-hydroxyflavone was given at a dose of 5 & 10 mg/kg 30 minutes prior to the i.p. injection of 0.6% acetic acid at 10 mL/kg body weight. The experiment measured the number of abdominal constrictions, trunk tuning, and limb extensions for each mouse, counting and classifying them as writhes. The data was collected from 5 minutes to 30 minutes following the acetic acid injection and expressed as a percentage of protection. Using the following formula, the % protection against acetic acid was determined [29].

% Protection = $[(N_c - N_t)]/N_c \times 100$

Were,

N_c- is number of writhing in control Rats

N_t- is number of writhings in test animals.

2.5 Biochemical Evaluation

2.5.1 Preparation of Serum Sample

At the end of the experimental protocol, blood samples were collected from animals under submaximal anesthesia using ketamine (50 mg/mL; 90 mg/kg body weight, i.p.) and xylazine (20 mg/mL; 10 mg/kg, i.p.) via retro-orbital puncture and transferred into gel tubes. The blood samples were allowed to clot at room temperature, followed by centrifugation to obtain serum. The separated serum samples were stored at –20 °C until further biochemical analysis. Animals were allowed to recover after the procedure in accordance with the guidelines of the Institutional Animal Ethics Committee. The overall sample preparation procedure was carried out according to the methodology described by Rasoul and Jwad (2022) [30].

2.5.2 Estimation of Lipid Peroxidase

The level of lipid peroxide in the serum was evaluated according to the method of Rasoul and Jwad, 2022 [30] and the absorbance was read at the wavelength (450 nm). The detailed method was given by Geetha and Geetha, 2014 [31]. Results were expressed as nmol MDA/mL serum.

2.5.3 Estimation of Glutathione Level

The level of glutathione in the serum was evaluated by using the assay kit supplied by BTLAB company and the ELISA device according to the method of Geetha and Geetha, 2014 [31]. And its absorbance was estimated at a wavelength of 412 nm. The detailed method was provided by author Rasoul and Jwad, 2022 [30]. GSH levels were expressed as µmol/mL serum.

2.5.4 Estimation of Superoxide Dismutase's Level

All of the group's animals had their blood samples taken in vials. Serum was separated and stored at –20 °C to estimate superoxide dismutase. The detailed method was mentioned by Kath and Gupta, 2006 [32], and Marklund S and Marklund G, 1974 [33]. SOD activity was expressed as U/mL serum, where one unit (U) represents the amount of enzyme required to inhibit 50% of superoxide radical formation.

2.6 Statistical Analysis

All the data were statistically analyzed, and charts were drawn using GraphPad Prism software (version 8.0.2.) (GraphPad Software, San Diego, CA, USA). Results were expressed as mean ± SD and considered statistically significant when $p < 0.05$. Statistical analysis between groups was performed using one-way and two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test or Sidak's multiple comparison test. The symbol "*" was used to compare standard or test groups with control where *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ denote the increasing levels of significance.

3. Results

3.1 The Effect of 7-Hydroxyflavone on Hot Plate Test

Oral administration of 7-hydroxyflavone at the low dose (5 mg/kg) produced a significant increase in the reaction time at 60 min ($p < 0.05$) & 120 min ($p < 0.01$) compared to the control group. In contrast, 7-hydroxyflavone at the high dose (10 mg/kg) & standard resulted in a sustained and significant increase in reaction time at 30, 60, 120 & 240 min ($p < 0.0001$) and exhibited an analgesic effect (Table 2 & Fig. 2).

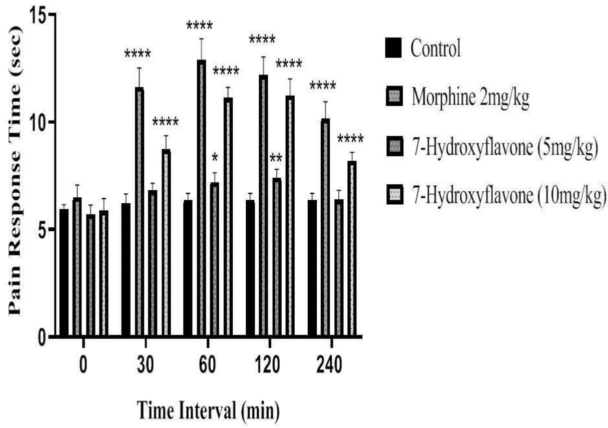

Fig. 2. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on pain response time (sec) in the hot plate test. Data are expressed as mean ± SD (n = 6 per group). Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple comparison test. *$p < 0.05$, **$p < 0.01$ and ****$p < 0.0001$ compared with the control group.

Table 2. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on pain response time (s) in the hot plate test.

Groups	Drugs	Response mean ± SD (in seconds)				
		Before	After			
			30 min	60 min	120 min	240 min
A	Normal saline (10 mL/Kg)	5.95 ± 0.20	6.21 ± 0.44	6.36 ± 0.31	6.36 ± 0.31	6.36 ± 0.31
B	Morphine (2 mg/Kg)	6.48 ± 0.58	11.61 ± 0.89****	12.88 ± 0.99****	12.18 ± 0.84****	10.15 ± 0.79***
C	7-Hydroxyflavone (5 mg/Kg)	5.7 ± 0.43	6.81 ± 0.33	7.2 ± 0.44*	7.4 ± 0.4**	6.38 ± 0.44
D	7-Hydroxyflavone (10 mg/Kg)	5.86 ± 0.57	8.73 ± 0.63****	11.11 ± 0.49****	11.23 ± 0.77****	8.18 ± 0.40****

Values are expressed as mean ± SD (n = 6 per group). Data were analyzed using two-way ANOVA followed by Dunnett's multiple comparison test. Values in parentheses indicate the increases in pain response time (sec) compared with the control group. Statistical significance was indicated as *$p < 0.05$, **$p < 0.01$ and, ***$p < 0.001$, ****$p < 0.0001$ Vs control group.

Table 3. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on tail flick latency (s) in the tail flick test.

Groups	Drugs	Response mean ± SD (in seconds)				
		Before	After			
			30 min	60 min	120 min	240 min
A	Normal saline (10 mL/Kg)	4.08 ± 0.26	4.4 ± 0.12	4.7 ± 0.23	4.76 ± 0.37	4.95 ± 0.18
B	Morphine (2 mg/Kg)	4.25 ± 0.25	8.08 ± 0.59****	9.85 ± 0.89****	10.43 ± 0.60****	9.4 ± 0.32****
C	7-Hydroxyflavone (5 mg/Kg)	4.1 ± 0.17	4.73 ± 0.22	5.05 ± 0.33	5.38 ± 0.41*	4.93 ± 0.29
D	7-Hydroxyflavone (10 mg/Kg)	4.15 ± 0.55	6.5 ± 0.67****	7.13 ± 0.47 ****	7.71 ± 0.46****	5.78 ± 0.34**

Values are expressed as mean ± SD (n = 6 per group). Data were analyzed using two-way ANOVA followed by Dunnett's multiple comparison test. Values in parentheses indicate the increases in tail flick latency (sec) compared with the control group. Statistical significance was indicated as *$p < 0.05$, **$p < 0.01$ and ****$p < 0.0001$ Vs control group.

3.2 Effect of 7-Hydroxyflavone on Tail Flick Latency

Oral administration of 7-hydroxyflavone at the low dose (5 mg/kg) produced a significant increase in the tail flick latency at 120 min ($p < 0.05$) compared to the control group. In contrast, 7-hydroxyflavone at a higher dose (10 mg/kg) produced a significant increase in tail flick latency at 30, 60, and 120 min ($p < 0.0001$) and 240 min ($p < 0.01$). However, the standard treatment exhibits a sustained increase in tail flick latency at the 30, 60, 120 & 240 min mark ($p < 0.0001$) compared to the control (Table 3 & Fig. 3).

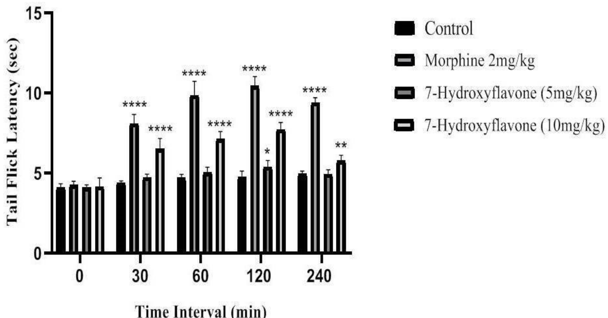

Fig. 3. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on tail flick latency (sec) in the tail flick test. Data are expressed as mean ± SD (n = 6 per group). Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple comparison test. *$p < 0.05$, **$p < 0.01$ and ****$p < 0.0001$ compared with the control group.

3.3 The Effect of 7-hydroxyflavone on Tail Withdrawal Latency Using Tail Immersion Test

Oral administration of 7-hydroxyflavone at the low dose (5 mg/kg) produced a considerable increase in the tail withdrawal latency at 30 min ($p < 0.01$) compared to the control group. In contrast, 7-hydroxyflavone at a higher dose (10 mg/kg) and the standard produced a significant and sustained increase in tail withdrawal latency at 30, 60, 120 & 240 min ($p < 0.0001$) compared to the control (Table 4 & Fig. 4).

3.4 The Effect of 7-Hydroxyflavone on Acetic Acid Induced Writhing Behavior

Oral administration of 7-hydroxyflavone produced a significant, dose-dependent reduction in acetic acid–induced writhing compared with the control group. 7-hydroxyflavone at the dose of 5 mg/kg produced a considerable reduction in writhing at 30 min ($p < 0.05$) compared to the normal control. However, treatment with 7-hydroxyflavone at 10 mg/kg, as well as the standard, resulted in a marked reduction in the number of writhes at the 30-min ($p < 0.0001$). These findings indicate that 7-hydroxyflavone exerts a potent dose-dependent antinociceptive effect in the acetic acid–induced writhing model (Table 5 & Fig. 5).

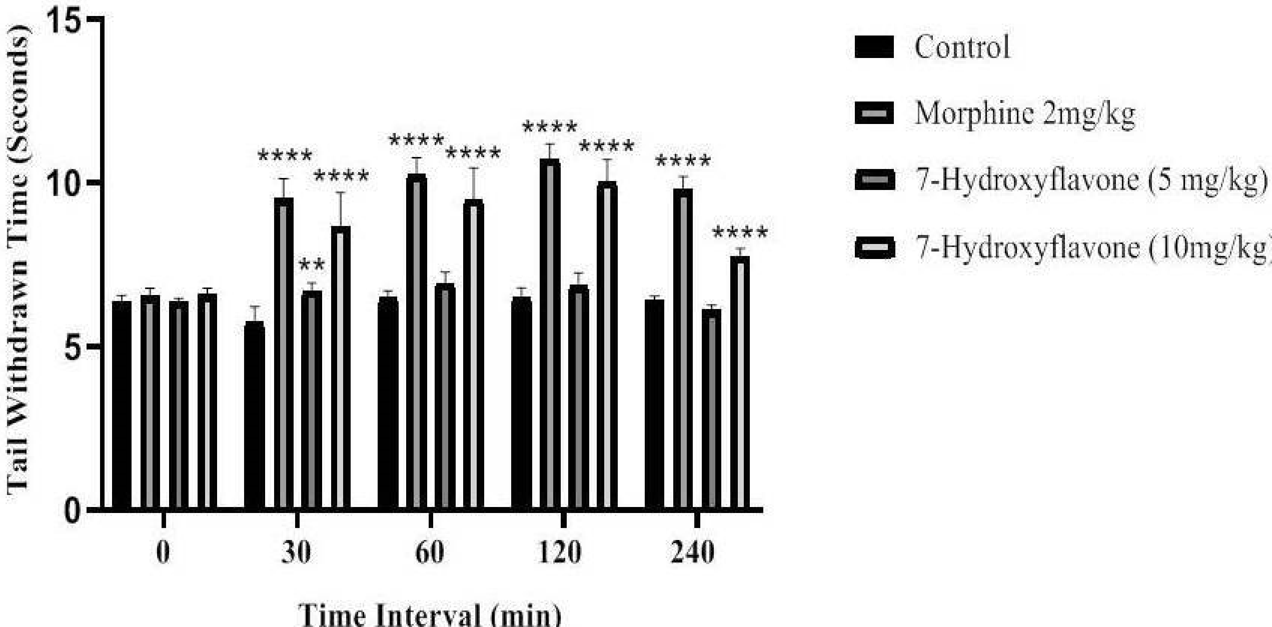

Fig. 4. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on tail withdrawal latency (sec) in the tail immersion test. Data are expressed as mean ± SD (n = 6 per group). Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple comparison test. **$p < 0.01$ and ****$p < 0.0001$ compared with the control group.

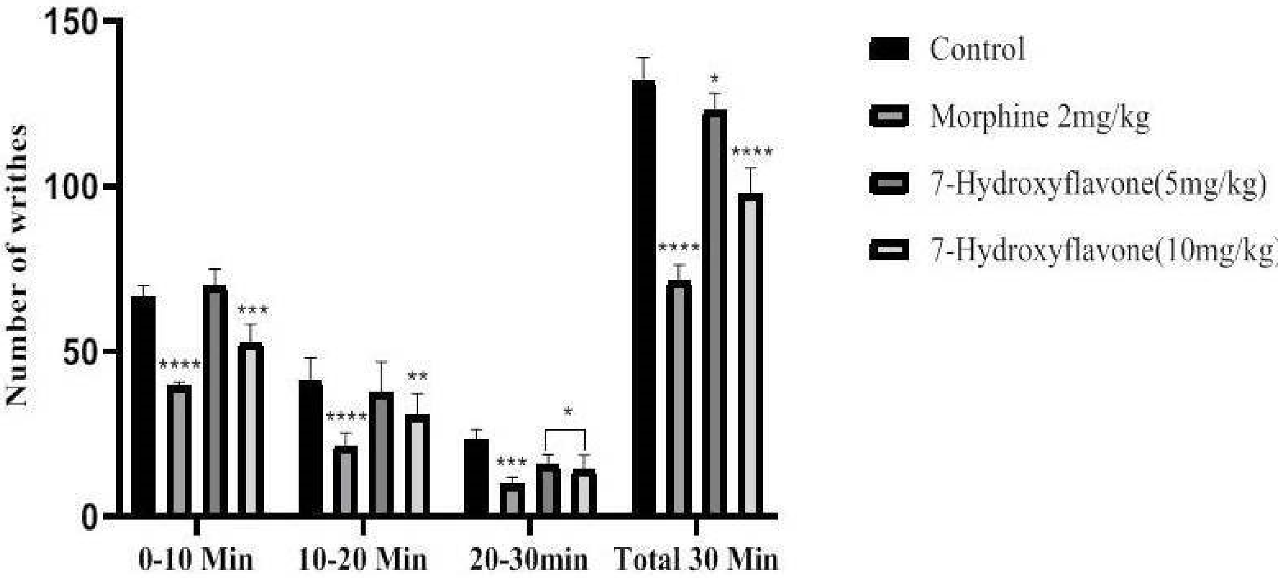

Fig. 5. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on writhing response in acetic acid-induced writhing test. Data are expressed as mean ± SD (n = 6 per group). Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple comparison test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ compared with the control group.

3.5 Effect of 7-Hydroxyflavone on Oxidative Markers (LPO, GSH and SOD)

Oral administration of 7-hydroxyflavone produced a significant, dose-dependent reduction in lipid peroxidation (LPO) levels compared with the control group. 7-hydroxyflavone at the dose of 5 mg/kg produced a considerable reduction in LPO levels ($p < 0.05$) compared to the normal control. However, treatment with 7-hydroxyflavone at 10 mg/kg, as well as the standard, resulted in a significant reduction in the LPO levels ($p < 0.0001$). These findings indicate that 7-hydroxyflavone ex-

Table 4. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on tail withdrawal latency (s) in the tail immersion test.

Groups	Drugs	Response mean ± SD (in seconds)				
		Before	After			
			30 min	60 min	120 min	240 min
A	Normal saline (10 mL/Kg)	6.36 ± 0.19	5.75 ± 0.47	6.48 ± 0.21	6.51 ± 0.27	6.4 ± 0.14
B	Morphine (2 mg/Kg)	6.53 ± 0.25	9.51 ± 0.62 ****	10.25 ± 0.52****	10.73 ± 0.46****	9.8 ± 0.40****
C	7-Hydroxyflavone (5 mg/Kg)	6.36 ± 0.10	6.7 ± 0.224**	6.93 ± 0.34	6.86 ± 0.38	6.13 ± 0.13
D	7-Hydroxyflavone (10 mg/Kg)	6.58 ± 0.19	8.66 ± 1.03****	9.50 ± 0.97****	10.03 ± 0.68****	7.76 ± 0.23****

Values are expressed as mean ± SD (n = 6 per group). Data were analyzed using two-way ANOVA followed by Dunnett's multiple comparison test. Values in parentheses indicate the increases in tail withdrawn time (sec) compared with the control group. Statistical significance was indicated as **$p < 0.01$ and ****$p < 0.0001$ Vs control group.

Table 5. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on writhing latency in the acetic acid induced writhing method.

Groups	Drugs	Writhing response mean ± SD			
		0–10 min	10–20 min	20–30 min	Total
A	Normal saline (10 mL/Kg) + Acetic acid	61.16 ± 7.90	41.16 ± 6.99	20.83 ± 2.85	123.15 ± 5.19
B	Morphine (2 mg/Kg)	51.16 ± 5.23****	29.16 ± 4.02****	16.5 ± 5.54***	96.83 ± 2.48****
C	7-hydroxyflavone (5 mg/Kg)	69.83 ± 5.15	37.66 ± 9.24	15.83 ± 2.99*	123.33 ± 4.88*
D	7-Hydroxyflavone (10 mg/Kg)	52.83 ± 5.60***	33.33 ± 5.60**	15.33 ± 5.46*	101.50 ± 1.64****

Values are expressed as mean ± SD (n = 6 per group). Data were analyzed using two-way ANOVA followed by Dunnett's multiple comparison test. Values in parentheses indicate the decreases in writhing response compared with the control group. Statistical significance was indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ Vs control group.

erts a potent dose-dependent antioxidant effect comparable to the standard drug (Fig. 6A). Furthermore, glutathione (GSH) content was significantly restored by oral administration of 7-hydroxyflavone at both doses ($p < 0.001$ for 5 mg/kg & $p < 0.0001$ for 10 mg/kg) compared to the control group. On the other hand, the standard also significantly restored the GSH content ($p < 0.01$) compared to the control (Fig. 6B). Similarly, superoxide dismutase (SOD) levels were significantly increased by 7-hydroxyflavone at the dose of 10 mg/kg and standard ($p < 0.0001$) compared to the normal control (Fig. 6C). Overall, 7-hydroxyflavone demonstrated a dose-dependent antioxidant effect, effectively mitigating oxidative damage and enhancing endogenous antioxidant defense mechanisms.

4. Discussion

The present study provides experimental evidence that 7-hydroxyflavone (7-HF) exerts dose-dependent analgesic activity through both central and peripheral mechanisms, with the higher dose (10 mg/kg) producing significant and sustained antinociceptive effects. 7-Hydroxyflavone (7-HF) effectively modulates analgesic activity through modulation of behavioral and oxidative parameters. These findings suggest that 7-HF possesses analgesic potential in experimental pain models and supports its involvement in both central and peripheral antinociceptive pathways.

Pain is a complex protective response that arises when tissue injury or dysfunction disrupts physiological homeostasis [1]. Analgesics are medications that target the peripheral or central nervous system to alleviate pain without causing substantial changes in consciousness [34]. Centrally acting analgesics raise the pain threshold and modify the physiological response. In contrast, peripherally acting analgesics block chemoreceptor impulses [35]. Analgesic activities were screened in pain-state models employing thermal stimuli like tail-flick and hot plate methods. Both approaches demonstrate centrally mediated antinociceptive responses, which focus generally on changes above the spinal cord level [36]. The tail-flick approach mediates a spinal reflex to a nociceptive stimulus, while the hot plate method is a supraspinal structured response [37].

The efficacy of 7-HF in central nociceptive models (hot plate, tail flick, and tail immersion tests) suggests possible involvement of supraspinal and spinal pathways, which are typically sensitive to centrally acting analgesics such as morphine. These models are sensitive to agents that modulate opioid receptors, ion channels, and descending inhibitory pathways.

7-hydroxyflavone was administered at low and high dose (5 and 10 mg/kg) and tested for antinociceptive potential utilizing hot plate, tail flick, and tail immersion models. The lower dose of 7-hydroxyflavone (5 mg/kg) did not show any analgesic effect. Steroids like calcitriol have a similar lack of analgesic effect at low dosages, suggesting that sub-therapeutic dosing may not modulate central nociceptive pathways [38]. However, 7-hydroxyflavone at 10 mg/kg increased reaction time, tail flick latency, and tail withdrawal time, indicating a significant improvement in pain threshold. Furthermore, the hot plate and tail flick experiments showed antinociceptive effects at 30 and 240

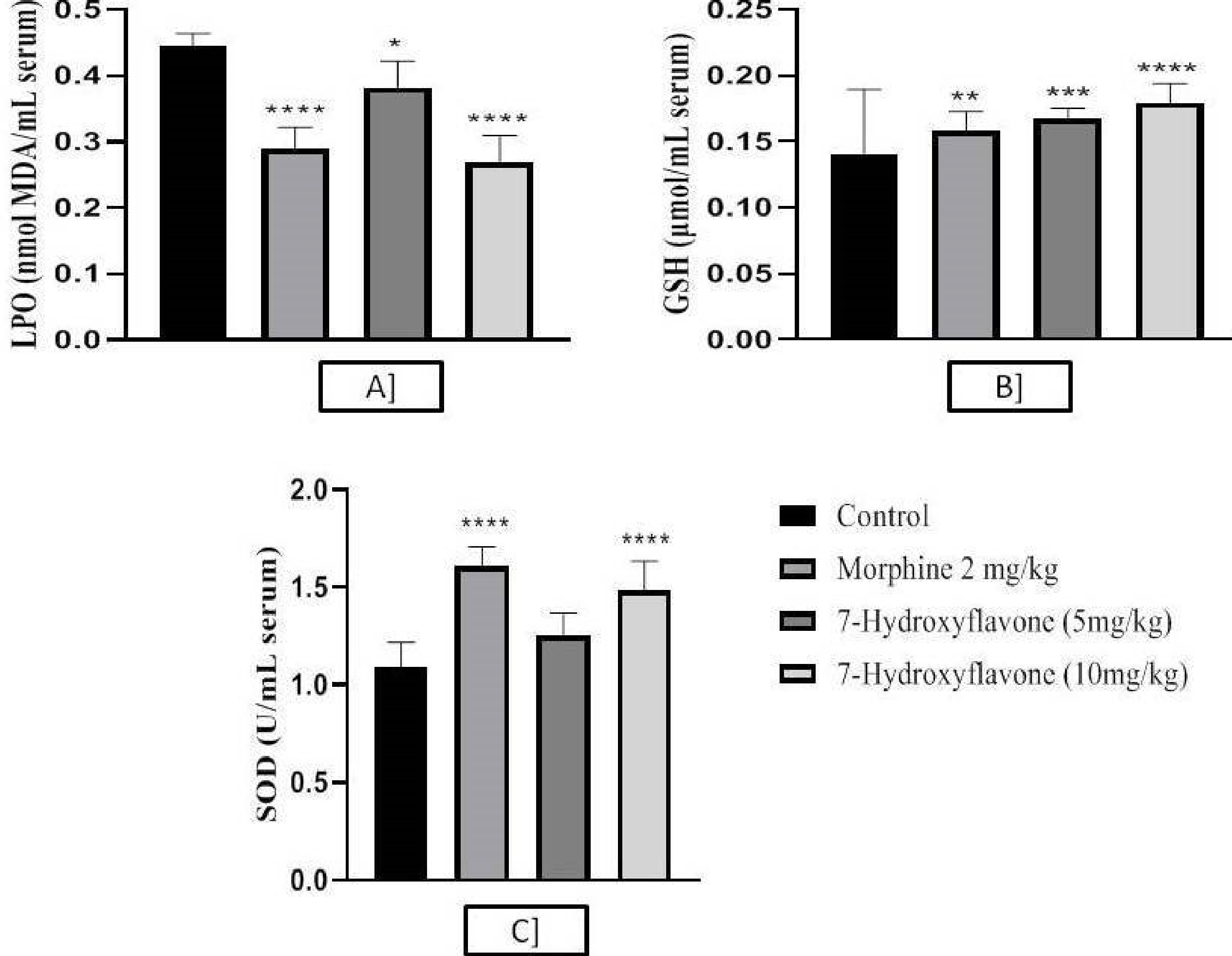

Fig. 6. Effect of 7-hydroxyflavone (5 and 10 mg/kg) on oxidative stress markers. (A) LPO activity, (B) GSH activity, (C) SOD activity. Data are expressed as mean $\pm$ SD (n = 6 per group). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison tests. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ compared with the control group.

min, suggesting a prolonged central analgesic effect. The consistent increase of pain threshold in the tail immersion test up to 240 minutes supports spinal and supraspinal neural mechanisms associated with 7-hydroxyflavone's higher dose analgesic profile; these results are correlate with earlier studies [1].

The acetic acid–induced writhing test was employed to evaluate the peripheral analgesic activity of the test compound. This model is widely regarded as a sensitive and reliable method for detecting antinociceptive effects, particularly for natural products and novel compounds that may remain inactive in other analgesic assays [39]. The peritoneal cavity gets irritated and stimulated by intraperitoneal acetic acid injection, which results in the production and release of endogenous inflammatory mediators, including histamine, serotonin, bradykinin, substance P, and PGs [40]. These different endogenous inflammatory mediators caused chemical-induced visceral pain, which is characterized by elongation of the body, extension of the forelimbs, and contraction of the abdominal muscles. The writhing test in response to acetic acid is therefore regarded as a model of visceral pain [41]. 7-hydroxyflavone dose-dependently showed significant peripheral analgesic activities by reducing the number of writhing compared to the control. The peripheral antinociceptive effect of 7-HF observed in acetic acid-induced writhing model may be associated with inhibition of inflammatory mediator release or reduced sensitivity of peripheral nociceptors in the peritoneal free nerve endings for chemically induced pain [41].

In this present investigation, the major strength lies in the integration of behavioral analgesic evaluation with oxidative stress assessment, allowing a mechanistic interpretation of the observed antinociceptive effects. It is well established that pain induction, particularly in chemically induced nociceptive models such as acetic acid–induced writhing, is associated with excessive generation of reactive oxygen species (ROS), leading to lipid peroxidation and depletion of endogenous antioxidant defenses [42,43]. Ele-

vated oxidative stresses have been shown to sensitize nociceptive pathways and exacerbate pain perception [44].

In the current study, administration of 7-hydroxyflavone (7-HF) significantly restored the levels of key endogenous antioxidants, including reduced glutathione (GSH) and superoxide dismutase (SOD), while concurrently reducing lipid peroxidation (LPO). These findings indicate a pronounced antioxidant effect of 7-HF under nociceptive conditions. The observed antioxidant restoration is consistent with previous reports demonstrating that flavonoids exert protective effects by enhancing cellular redox balance and suppressing oxidative damage [45,46]. Notably, accumulating evidence suggests that flavonoids activate the nuclear factor erythroid 2–related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling pathway, which plays a central role in regulating antioxidant enzyme expression and maintaining cellular homeostasis [47]. Activation of the Nrf2/HO-1 axis has been linked to reduced oxidative stress–mediated neuronal sensitization and attenuation of pain signalling [48]. Therefore, the antioxidant effects observed in the present study may contribute at least in part, to the antinociceptive activity of 7-HF potentially through modulation of oxidative stress–dependent nociceptive pathways.

Morphine was employed as a standard due to its well-characterized and robust analgesic efficacy across multiple nociceptive models. Morphine's well-known side effects sedation, respiratory depression, constipation, tolerance, and dependence limit its clinical use. Flavonoid-based drugs like 7-HF may reduce opioid-related side effects by acting as non-opioid analgesics. The tested doses of 7-HF did not show behavioral abnormalities. The limitation of the current study is orally administered 7-HF and intraperitoneally administered morphine may differ in bioavailability and latency to peak effects. Morphine was chosen as a reference standard due to its well-characterized and substantial analgesic efficacy in multiple nociceptive models. Therefore, the comparison assessed relative pharmacodynamic efficacy, not pharmacokinetic equivalency. Oral conventional analgesics, such as aspirin or other non-steroidal anti-inflammatory drugs, might provide a more direct route-matched comparison in future investigations.

The antinociceptive effects of 7-HF at the tested dose showed measurable efficacy in several parameters, although direct equivalence with morphine can not be concluded due to differences in route of administration and pharmacokinetics. Overall, this study supports the potential of 7-HF as a promising analgesic candidate with antioxidant properties, warranting further pharmacological, mechanistic and translational stuudies to confirm its therapeutic relevance. The efficacy of 7-HF at 10 mg/kg doses further provides scope for its dose optimization. More study is needed to be required for the confirmation of the precise mechanism of the test drug as an analgesic potential and factors associated with pain and analgesia.

5. Conclusion

In conclusion, the 7-hydroxyflavone (7-HF), particularly at a dose of 10 mg/kg, demonstrated significant analgesic activity in the experimental rat model. The treatment with 7-HF effectively attenuated both central and peripheral nociception, as evidenced by the hot plate, tail flick, tail immersion, and acetic acid-induced writhing tests. In addition, 7-HF showed a favorable safety profile and was associated with improvement in antioxidant parameters, suggesting a potential link between its analgesic and antioxidant effects. The observed analgesic efficacy of 7-HF may be attributed to its multi-target mechanism of action. However, further studies are required to confirm its efficacy, safety, and therapeutic potential in the management of pain.

6. Summary

In this study, Rats were used to investigate the analgesic properties of 7-hydroxyflavone compared to morphine and a control group. The experimental animals were subjected to various tests to measure pain perception, including the hot plate, tail flick, and tail immersion tests for central mechanisms, and acetic acid-induced writhing for peripheral mechanisms. 7-hydroxyflavone were tested at low and high dose (5 mg/kg and 10 mg/kg), with the higher dose it showing significant analgesic effects compared to the lower dose and control groups. Moreover, the higher dose of 7-hydroxyflavone improved pain thresholds and reduced writhing responses in the experimental animals. Notably, in this study 7-hydroxyflavone treatment rats showed more significant restoration of antioxidant enzymes level like glutathione and superoxide dismutase and by reducing lipid peroxidation level that indicates inhibition of oxidative stress to pertaining its analgesic effect via central and peripheral mechanism.

Abbreviations

7HF, 7 hydroxyflavone; SOD, Superoxide Dismutase; LPO, Lipid Peroxidation; GSH, Glutathione.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

ADT and SVK designed and conducted behavioural experiments. SVK performed statistical analysis. SPB, SBK, MRJ, and SPJ assisted in data compilation and manuscript drafting. ADT prepared the initial manuscript draft. SPJ supervised the work and revised the manuscript to its final version. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was done in accordance with the committee for the purpose of control and supervision of experimental animals (CPCSEA) guidelines after approval of the experimental protocols by the institutional animal ethics committee (IAEC) Rajarshi Shahu College of Pharmacy, Buldana. Project Proposal No. IAEC 1865/24-25/P-11.

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Conflict of Interest

The authors declare no conflict of interest.

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