

Preclinical assessment of central and peripheral anti-nociceptive effect of taraxerol acetate

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ABSTRACT

Background: Successful management of pain remains a main clinical problem due to adverse effects caused with prolonged use of analgesics. This has motivated ongoing research into natural compounds that may possess therapeutic properties. A penta cyclic triterpene, Taraxerol acetate, has been reported to hold numerous biological activities; however, its analgesic properties have not been expansively appraised. The current study aimed to investigate using well-established experimental pain models in mice, the peripheral and central analgesic effects of taraxerol acetate.

Methods: Central analgesic activity was determined by the hot plate test while peripheral analgesic activity was assessed using the acetic acid-induced writhing assay, Taraxerol acetate was administered orally at doses of 25 and 50 mg/kg. As reference drugs, Aspirin (150 mg/kg) in the writhing and tramadol (5 mg/kg) in hot plate models were used. To evaluate the analgesic effects of taraxerol acetate, the number of abdominal writhes, percentage inhibition, and reaction time were recorded and analyzed.

Results: Taraxerol acetate significantly reduced the number of acetic acid-induced writhes compared with the control group. The effect was dose dependent. The lower dose produced 51.08% (p value <0.05) inhibition of writhing, while the higher dose produced 77.57% (p value <0.01) inhibition. In the hot plate test, taraxerol acetate significantly increased the reaction latency. The 50 mg/kg dose showed the highest analgesic effect (p value <0.01). At some observation times, the analgesic effect of the higher dose was close to that of the standard drug. These findings suggest that taraxerol acetate may act through both peripheral and central pain-modulating mechanisms.

Conclusion: Significant analgesic activity of taraxerol acetate against chemically induced pain and increased tolerance to thermal pain was identified in experimental. However, further studies are required to investigate its mechanism of action in detail and to evaluate its potential therapeutic role.

Keywords: Analgesic activity, Hot Plate Test, Taraxerol Acetate

This article may be cited as: Rahman UU, Khattak N, Ubaidullah, Ubaidullah S, Khan MA. Preclinical assessment of central and peripheral anti-nociceptive effect of taraxerol acetate. *Int J Pathol*; 24(3):342-50. DOI: <https://doi.org/10.59736/IJP.24.03.1105>

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Introduction

Pain is the most prevalent health concern world-wide. Disease related chronic pain such as secondary to inflammation, cancerous issues, traumatic pain, neurological pain or any metabolic disorders take a toll on the

patient's both, mental and physical health and their quality of life. (1) Despite advances in pain management, the development of safe and effective analgesic therapies with minimal adverse effects remains an important clinical task.

Non-steroidal anti-inflammatory drugs (NSAID), the commonly used over the counter available, pain relieving drugs, give rise to grave side effects which include gastrointestinal ulcers, renal impairment, and cardiovascular complications. (2) Opioid analgesics used for a long time period lead to the development of dependence, risk of misuse, increased tolerance and most dangerously respiratory depression especially in the elderly patients. Thus opioid analgesic drugs are highly unsuitable for prolonged pain management. (3) These limitations highlight the ongoing need to identify natural, safer, and more effective alternative analgesic agents with improved safety profiles for routine clinical use.

Plant-derived natural products have demonstrated considerable therapeutic potential, owing to their diverse biological activities and are increasingly recognized as valuable candidates for the management of various diseases, including pain. (4,5) Phytochemicals, particularly triterpenoids, have attracted considerable attention owing to their broad spectrum of pharmacological activities. (6) This diverse class includes compounds such as taraxerol acetate, lupeol, ursolic acid, oleanolic acid, and several other naturally occurring triterpenes. Such natural compounds have exhibited anti-pathogenic, anti-inflammatory, cyto protective, hepato protective, insulin sensitizing and analgesic activities. Their beneficial effects are primarily attributed to their ability to modulate inflammatory mediators, reduce oxidative stress, and regulate intracellular signaling pathways

involved in the pathogenesis of various diseases. (7)

Taraxerol acetate, an acetylated derivative of taraxerol, is an oleanane-type pentacyclic triterpenoid that has been isolated from several medicinal plant species, including *Artemisia roxburghiana*, *Taraxacum officinale* and *Ambrosia Artemisiifolia*. Taraxerol acetate has demonstrated a multi-targeted and multi-faceted therapeutic profile, including analgesic effect. (8) These diverse biological properties suggest that taraxerol acetate is a promising natural therapeutic agent, particularly for the management of inflammatory and pain-related disorders.

In this study, we investigated both, central and peripheral analgesic effect of taraxerol acetate with acetic acid-induced writhing and hot plate tests. The findings of this study provide experimental evidence supporting the analgesic potential of taraxerol acetate and establish a foundation for future mechanistic and preclinical investigations.

Methods

Taraxerol acetate, the test compound was isolated from a plant, *Artemisia roxburghiana* was found in Hazara University campus, Mansehra, Pakistan. Its aerial parts were collected. At the Herbarium of Hazara University, the authenticated voucher specimen (no 3486) was deposited.

These plant materials were dried in shade and then powder was formed by grinding, the weight of which was 17 Kg (doi.org/10.3109/14756366.2015.1047358). The study was approved from the Institutional Research Committee of Khyber Medical College with approval no. 170/DME/KMC.

Aspirin and tramadol were used as the standard drugs for evaluating peripheral and central analgesic activities, respectively. Other chemicals including normal saline and acetic acid were of analytical grade.

This experimental animal model utilized health Swiss albino mice (either sex) of weight 25–35 g which were obtained from PCSIR laboratories Peshawar. The animals were housed under standard laboratory conditions at a temperature of $22 \pm 2^\circ\text{C}$ with a 12-hour light/12-hour dark cycle and were provided with a standard laboratory diet and water ad libitum. Before the experiments, the animals were acclimatized to the laboratory environment for one week. Resource Equation Method ($DF=N-k$, range 10–20) was used for the sample size calculation. It showed $n=4-6$ per group for our study; thus, we used $n=6$ for writhing test and $n=5$ for hot plate test (9)

We allocated animals randomly to the experimental groups using a computer-generated randomization sequence. Allocation was further stratified by body weight in order to minimize confounders. Also the order of testing was randomized daily. The investigators responsible for outcome assessment (writhing counts and hot plate latencies) were blinded to treatment group.

Acetic acid-induced writhing test was utilized to assess for the peripheral analgesic effect of taraxerol acetate. (9) Mice were categorized in four groups randomly ($n=6$). The control group I received normal saline. Groups II received oral TA at dose 25 mg/Kg body weight while group III at 50 mg/Kg. Aspirin as oral dose of 150 mg/Kg body weight was administered to group IV as the standard drug. Twenty minutes later, all animals were injected intra peritoneally with acetic acid in the dose 10 mL/kg for induction of abdominal writhing. The number of abdominal constrictions (writhes) was recorded for 20 minutes and the percentage inhibition of writhing was calculated using the following formula:

Percentage inhibition = $\left[\frac{\text{Mean number of writhes in the control group} - \text{Mean number of writhes in the treated group}}{\text{Mean number of writhes in the control group}} \right] \times 100$

The hot plate method was used to assess the central analgesic activity of TA. (10) Swiss female albino mice were randomly categorized in four groups ($n=5$). Female mice were selected due to their higher baseline sensitivity in the hot plate test, and the sample size was reduced to 5 mice per group based on the statistical efficiency of the repeated measures design. Normal saline was given orally to the control group, while the treatment groups received taraxerol acetate orally at doses of 25 mg/kg and 50 mg/kg. The standard group received tramadol (5 mg/kg, orally).

The temperature of the hot plate apparatus was maintained at $55 \pm 0.5^\circ\text{C}$ throughout the experiment. The reaction time was recorded at 30, 60, 90 and 120 minutes following drug administration. Measurement of the response time commenced after the animals were put on hot plate, till it exhibited first physical action of paw withdrawal, jumping or paw licking. The animal was removed from the hot plate if no response initiated within 30 seconds.

SPSS 25 version was used for statistical analysis of the data. Writhing test results are presented as mean $\pm$ standard deviation (SD). The differences between the groups were checked using one-way ANOVA, followed by Dunnett's multiple comparison test to compare each treated group with the saline control group. The results of the hot plate tests are expressed as means $\pm$ SD. These data were analyzed using two-way repeated-measures ANOVA, followed by Dunnett's multiple comparison test. A P value of less than 0.05 was taken as statistically significant.

Results

The acetic acid-induced writhing model was used to study the peripheral analgesic activity of taraxerol acetate. As shown in Table 1,

during the 20-minute observation period, taraxerol acetate treatment significantly reduced the number of abdominal writhes and the effect was increased with dose.

Acetic acid-induced writhing in mice was significantly reduced by taraxerol acetate. The analgesic activity increased with increasing dose from 25 to 50 mg/Kg.

The comparison of the experimental conditions was carried out using independent student's t test. The comparison of 25 mg TA with Aspirin showed a significantly higher effect of aspirin with a p value of <0.001. The comparison of 50 mg TA with Aspirin did not show significant result (p value 0.06).

Table1. Acetic Acid-Induced Writhing in Mice with Increasing Dose of Taraxerol acetate

Treatment	Dose (mg/kg)	Number of Writhes (mean $\pm$ SD)	% Inhibition	Comparison vs. Aspirin (150 mg/kg) p-value
Saline	—	27.6 $\pm$ 2.12	—	
Taraxerol Acetate	25	13.5 $\pm$ 1.29*	51.08	<0.001**
Taraxerol Acetate	50	6.19 $\pm$ 1.57**	77.57	0.06
Aspirin	150	3.31 $\pm$ 2.03**	88	

P < 0.05 (*), *P* < 0.01 (**) compared with the saline control group.

Central analgesic effect of taraxerol acetate was assessed with hot plate test. Table 2, shows that saline-treated control group exhibited the lowest reaction latency throughout the study. Treatment with taraxerol acetate increased the reaction latency, and the increase was greater at the higher dose. Tramadol (5 mg/kg) produced the highest reaction latency during the study. Taraxerol acetate at 50 mg/kg also showed a marked increase in reaction latency, particularly at 60 minutes, indicating central analgesic activity.

The intergroup comparison with the standard drug, tramadol (5 mg/kg)

demonstrated a significantly higher analgesic activity at 60 min compared to both taraxerol acetate 25 mg/kg (*p* < .001) and 50 mg/kg (*p* = <0.05). By 90-min interval, the analgesic effect of both TA treatment groups sustained efficiently, with no statistically significant difference when compared directly to the tramadol group. Taraxerol Acetate at 50 mg/kg displayed prolonged efficacy comparable to tramadol throughout the middle-to-late observation window (30 to 90 min), highlighting its potent dose-dependent analgesic capacity.

Table 2. Taraxerol Acetate Effect on Hot Plate Latency in Mice

Treatment	Dose (mg/kg)	30 min (s)	60 min (s)	90 min (s)	120 min (s)
Saline	—	12.86 $\pm$ 0.93	12.16 $\pm$ 0.69	12.37 $\pm$ 0.71	12.02 $\pm$ 0.47
Taraxerol acetate	25	14.19 $\pm$ 1.47	17.37 $\pm$ 1.41* ##	17.28 $\pm$ 1.31*	12.22 $\pm$ 0.78
Taraxerol acetate	50	17.41 $\pm$ 2.13*	21.36 $\pm$ 1.33** #	19.57 $\pm$ 2.62**	14.18 $\pm$ 4.9
Tramadol	5	21.00 $\pm$ 5.10**	24.28 $\pm$ 1.16**	18.22 $\pm$ 1.07**	12.17 $\pm$ 0.89

P < 0.05 (*), *P* < 0.01 (**) compared with the saline control group.

P < 0.05 (#), *P* < 0.001 (##) compared with the taraxerol acetate group.

Discussion

Pain is a significant medical issue globally. It also accounts for the leading cause of disability. The search for safer and more effective analgesics from natural sources has therefore gained considerable attention in recent years. This study assessed the peripheral and central analgesic activity of TA with established animal experimental models. TA produced substantial analgesic effect in both models, and in a dose-dependent way. The acetic acid induced writhing test, the compound markedly reduced the number of abdominal writhes, indicating effective peripheral analgesic activity. TA significantly prolonged reaction latency in the hot plate test, thus suggesting association of central pain-modulating system. Higher dose of 50 mg/kg produced increased analgesic effect than lower dose i.e 25 mg/kg, however; the efficacy did not exceed that of the standard analgesics, aspirin and tramadol. Collectively, these findings indicate that taraxerol acetate possesses appreciable anti nociceptive activity and may influence both peripheral and central mechanisms involved in pain perception (11,12).

The significant reduction in writhing observed with taraxerol acetate in the present study is consistent with findings for other plant-derived triterpenoids. Similar results were observed in a study on *Rhododendron arboreum* bark containing taraxerol, which produced 82% inhibition in the same model. (13) Similarly, ethanolic fraction of *Launaea fragilis* exhibited significant analgesic results in acetic acid-induced writhing test. (14)

Our findings indicate that, in addition to its peripheral analgesic effects, taraxerol acetate may influence central mechanisms involved in pain perception. These findings are in line with those of study performed on Cannabis-derived terpenes by Alfieri et al (15). They

reported that *linalool* and β -*caryophyllene* compounds produce significant analgesia in writhing and hot plate models through multi-target mechanisms. Similarly, Chen et al. (16) observed that *grayanane diterpenoids* from *Rhododendron molle* show strong antinociceptive effects in writhing test, at substantially lower doses than taraxerol acetate. This may reflect differences in chemical class as well as target affinity. In contrast to the diterpenoids, that primarily act peripherally, TA also prolonged hot plate latency, which indicates its additional central activity. While its potency was lower than the standard drugs, the dual peripheral and central effects, coupled with its favorable safety profile as a naturally occurring triterpene, support further investigation of taraxerol acetate as a potential analgesic agent. The results of our study showed a positive difference between the effects of taraxerol acetate and other generic crude extracts. where the latter show low or volatile peak scores, TA in 50 mg/kg dose secured increased anti-nociception, almost close to tramadol, hence confirming its advanced pharmacodynamic profile with a more sustained prolonged central modulatory effect. (17-18)

The present study provides experimental evidence supporting the analgesic potential of taraxerol acetate using two complementary animal models that evaluate both peripheral and central nociceptive mechanisms supporting the evidence which suggest that naturally occurring penta cyclic triterpenes represent promising candidates for the development of novel analgesic and anti-inflammatory agents. (19,20)

Limitations of study: Nevertheless, the present study has certain limitations. The underlying molecular mechanisms responsible for the observed analgesic effects

were not investigated and biochemical markers of inflammation or oxidative stress were not evaluated. In addition, pharmacokinetic, toxicity and long-term safety studies were beyond the scope of the present investigation.

Future Recommendations: Further studies involving molecular targets, receptor-binding assays, gene expression analyses and chronic toxicity evaluations are warranted to better understand the mechanism of action, its safety profile and any pharmacological potential before the TA is clinically applied.

Conclusion

This study demonstrated that taraxerol acetate possesses significant peripheral and central analgesic activities in experimental animal models. Taraxerol acetate reduced acetic acid-induced writhing in a dose-dependent manner and increased reaction latency in the hot plate test. Although its effect was lower than that of the standard drugs, it showed good analgesic potential as a natural compound.

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ISTORY	
Date received:	28-07-2026
Date sent for review:	24-09-2026
Date received reviewers comments:	30-09-2026
Date received revised manuscript:	30-09-2026
Date accepted:	30-09-2026

CONTRIBUTION OF AUTHORS	
AUTHOR	CONTRIBUTION
Conception/Design	UR,SD,NK,UU,SU,MAK
Data acquisition, analysis and interpretation	UR,SD,NK,UU,SU,MAK
Manuscript writing and approval	UR,SD,NK,UU,SU,MAK
All the authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed.	