Research Article

Comparative Analgesic Effect of *Portulaca oleracea* L. Extracted Apigenin and Diclofenac in Mice

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Abstract | This article aims to determine the analgesic impact of Apigenin and Diclofenac in mice model. In the beginning, 84 male albino mice were selected, acclimated, and categorized into four equal groups: The 1st tested by the hot plate, the 2nd treated with acetic acid, the 3rd examined by application 10 µl of 2% formalin solution and the 4th was examined by the tail-flick test. Within each experiment, the study mice were subdivided equally into three subgroups; the 1st was administered only distilled water (control), the 2nd was drenched with apigenin (50 mg/Kg B.W.) orally, and the 3rd was drenched only diclofenac (0.71 mg/kg B.W). The hot plate's outcomes showed that the animals treated with apigenin significantly increased in time than with diclofenac. The number of writhes in both apigenin and diclofenac-treated groups has been significantly reduced in the acetic acid test when compared to the control. The formalin test revealed that both apigenin and diclofenac-treated groups reported significantly lower flinches and licks than the control. For the tail-flick test, the results have significantly increased in withdrawal time in apigenin and diclofenac-treated groups than with the control; with the ability of apigenin to relieve the pain when compared with diclofenac.

Keywords | Herbal therapy, Hot plate, Acetic acid, Formalin solution, Tail-flick test, Iraq

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INTRODUCTION

As a complicated phenomenon, pain is challenging to measure and characterize, with a common description as a disagreeable sensory and emotional experience connected to a current or potential tissue injury (Al-Tahan *et al*., 2012; Lovich-Sapola *et al*., 2015). The International Association for the Studies of Pain (IASP) stated that pain is classified in several ways according to: (1) the affected region in the body (such as the head and viscera), (2) the occurrence pattern, (3) the duration of the type of disease or injury that is causing it (e.g., Fibromyalgia, persistent post-surgical pain, cancer, pain of neuropathic, migraine and headache, or pain of facial). However, it recommended that only symptoms, causes, and syndromes can be used for categorization. As a result, there are three distinct types of pain that have been identified on a global scale: nociceptive pain, neuropathic pain, and inflammatory pain (Yam *et al*., 2018). Nociceptive pain is a neurological mechanism reflex that encodes and processes the noxious stimulation (Prescott *et al*., 2014). Elevation reflex to painful stimulation is known as hyperalgesia, and the pain is felt as a result of non-noxious stimulation (allodynia). Neuropathological pain (NP) and peripheral neural injury are combined with neuronal inflammation at the regional level, which is correlated with increased capillary

permeability caused by mast cell activation, followed by neutrophil and macrophage recruitment (Smart *et al*., 2010). Moreover, the alleviation of hypersensitivity following nerve injury can be achieved through the selective inhibition of inflammatory cell activation and/or recruitment, the pharmacological reflex of oxidative effect, and the expression of pre-inflammatory cytokines (Hsieh *et al*., 2020). The powerful painkiller sedation is known as an opioid (Cogan *et al*., 2020). Commonly, Non-steroidal anti-inflammatory medications (NSAIDs) are utilized to deal with pain, have a number of undesirable effects, such as renal and hepatic dysfunction, and thus restrict their therapeutic efficacy in pain treatment (Hasan *et al*., 2014; Pirkulashvili *et al*., 2017). However, these drugs provide only temporary relief for neuropathic in a small percentage of ill people and frequently cause side effects affecting the central nervous system (CNS), which are severe and dose-limiting (Furlan *et al*., 2006). Diclofenac is an NSAID that is regrettably linked to serious gastrointestinal adverse effects; these could occasionally develop life-threatening conditions that result in gastrointestinal tract ulcers and bleeding (Kadhim and Kubba, 2020). *Portulaca oleracea* Family Portulacaceae, also known as The World Health Organization, ranks purslane (Portulaca oleracea Family Portulacaceae), an annual herb, among the most frequently utilized medicinal plants (Srivastava *et al*., 2021). The utilization of plant raw materials, in conjunction with suitable active substances and the correct dosage, has emerged as a more advantageous and safer approach to treating numerous diseases, as the worldwide trend towards substituting hazardous chemicals with plant-based alternatives in pharmaceutical preparations has accelerated (Ahmad *et al*., 2017; Hasan, 2019). The herb P. oleracea grows annually and is widely distributed around the world; it has long been used to treat a number of ailments, P. oleracea is linked to various medicinal benefits, such as anti-inflammatory, anti-fever, and analgesic actions (Allahmoradi *et al*., 2018). The primary and secondary metabolites in P. oleracea include alkaloids, terpenes, coumarins, flavonoids, organic acids, and other ingredients (Syed *et al*., 2016), which have strong antibacterial, anti-inflammatory, analgesic, anti-tumor, anti-oxidation, immune enhancement, and anti-cough effects (Jiang *et al*., 2021). Flavonoids, which are classified as plant secondary metabolites characterized by their polyphenolic structure, are an essential category of natural products. Widely, they can be found in certain beverages, vegetables, herbs, and fruits. Flavonoids are potential anti-allergic molecules, antimicrobial, antiviral, analgesic, antioxidant and anti-inflammatory. They are classified as one of the most natural compounds that reduce the incidence of heart disease (Al-Okaily, 2009). An important category of natural products consists of flavonoids, which are secondary plant metabolites characterized by a polyphenolic structure. These compounds are widely distributed in some drinks, herbs, vegetables, and fruits. Apigenin is one of the flavonoids that contribute significantly to the nutraceutical content of the diet. A flavonoid with the chemical name 4′, 5, 7, trihydroxy flavone and the molecular formula C15H10O5. Apigenin is a member of the structural class of flavones, has MW 270.24 (molecular weight), and in its pure state, it structurally forms yellow needles. Apigenin possesses an antioxidant property and immunomodulation effect (Rakha *et al*., 2022). The current article aims to extract the apigenin from the *Portulaca Oleracea* L. plant and assess the analgesic effect of apigenin with Diclofenac in the mice model.

MATERIALS AND METHODS

Plant collection and powder preparing

Mature wild *P. oleracea* plants were harvested manually from different areas of Baghdad. After collection, plants were combined to create at least one sample. Before extraction, the leaves were dried, powdered with a mortar and pestle, and kept at room temperature.

Extraction and preparation of apigenin

A total of 35 gm of the powder was put in a Soxhlet extractor with 350 ml of ethanol: water (70: 30) solvent at 60°C for 6 hours. The extract was kept in the dark at 4°C until further use (Nayaka *et al*., 2014). Using the HPLC method, we isolated Apigenin from *P. oleracea* L. described by Radovanovic *et al*. (2015). The apigenin dilutions were generated by accurately measuring 20 mg of apigenin and subsequently adding distilled water to get a final volume of 4 ml. The concentration at the end of the experiment was measured, and the resulting dose was determined to be 0.1 ml /10 gm of mice body weight, equivalent to a dose of apigenin at 50 mg/kg.

Preparation of diclofenac

The dilutions of diclofenac were produced by adding distilled water to a volume of 100 ml after weighing 7.1 mg of diclofenac. The final concentration was documented, and the corresponding final dose was computed to be 0.1 ml/10gm of mouse body weight, which is equivalent to the diclofenac dose of 0.71 mg/kg.

Experimental design

Three months old age of 84 male albino mice with 27±2 gm weight were purchased. The mice are housed in stainless steel wire mesh cages, with a bedding of wood shavings, and maintained at a temperature of 25±3°C and 12/12 hours of light/dark cycle. At all times, water and food were given except before drug administration. Then, the mice were divided randomly and equally into four experiments:

1. The hot plate examined the 1st group at a temperature

of 55±1°C, and the reaction times of each animal towards thermal stimulus were recorded for jumping responses or paw licking. After administering test materials, all the animal groups will put their paws on the hot plate. Reaction time is measured in seconds for actions like jumping or licking the front paw. There is a 15-second time limit to prevent paws from getting hurt (Eddy and Leimbach, 1953).

2. The 2nd group was treated with acetic acid 1%, 0.25 ml I.P. After 30 minutes of giving the test compounds, the analgesic effect was measured by counting the number of writhes for a maximum of ten minutes. A tightening of the abs shows Writhe, full back leg expansion, and body stretching (Collier *et al*., 1968).
3. The 3rd group was examined by application of 10 µl of 2% formalin solution subcutaneously into the plantar surface of the right hind foot (Figure 2). Two pain-related behaviors were targeted to detect the pain response: licking the injected paw and flinching. The pain response measurement was carried out immediately.
4. The 4th group was examined by the tail-flick test, D'amour and Smith (1941) explained it as placing the tip (last 1-2 cm) of an animal's tail on a radiant heat source (warm water at a temperature of 55 1°C) and recording the animals' initial reaction to the heat. From the flicking response (heat source), the tail withdrawal has been used as the endpoint. Those animals that exhibited a fluttering response within three to five seconds were chosen for the work. A cut-off period of 15 seconds was suggested to prevent tail damage. The withdrawal time has been measured with warm water 30 minutes after the medicines were administered (Figure 3).

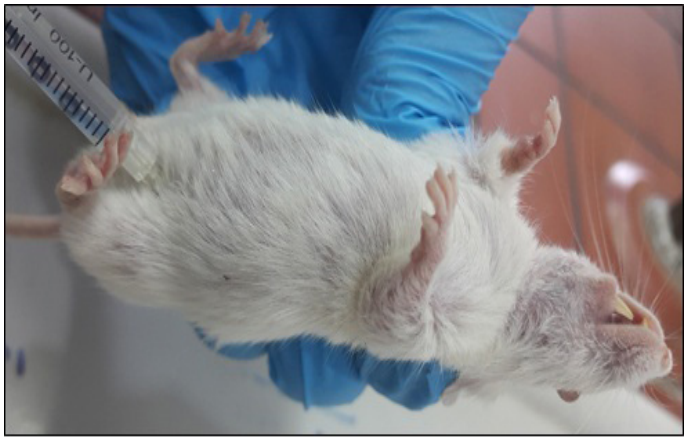

Figure 1: Injection of acetic acid intraperitoneally to induce pain in mouse.

The study animals were subdivided equally i n to four groups: the 1st as control (treated only with distilled water), the 2nd as experimental group 1, in which the mice were treated only with apigenin at a dose of 50 ml/kg, B.W (Saleh, 2010; El-Shoubaky *et al*., 2016; Sadraei *et al*., 2017; Jaafar *et al*., 2018; Mondal *et al*., 2022). Then, in the 3rd as experimental group 2, the mice were treated only with diclofenac at a dose of 0.71 mg/kg B.W. (Jaafar *et al*., 2018).

Figure 2: Injection of formalin in the planter surface of the paw to induce pain in mouse.

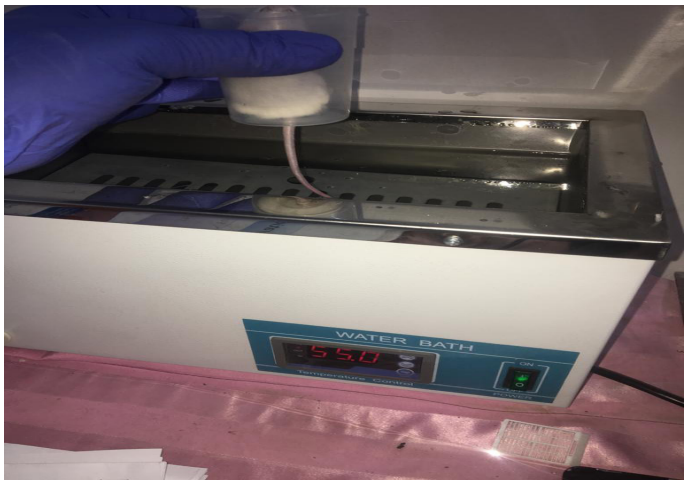

Figure 3: Placing the tip of the tail on the water bath as heat source.

Statistical analysis

To determine the impact of the parameters, the Statistical Analysis System (SAS) (version 2018) programmer has been utilized in the data analysis. A significant comparison of means was conducted in this study by employing the least significant difference (LSD) test (ANOVA) (Gharban, 2023).

RESULTS AND DISCUSSION

Extract characteristics and apigenin concentration

Extraction of *P. oleracea* with ethanol 70% by the soxhlet method gave a dark green color solution extract.

Sampling concentration= Sample area/ Standard area of x standard concentration (ppm) Sampling concentration in 100 ML = 3036.491 / 11434.438 Ĭ 10 ppm

The concentration of the sample in 100 ML = 2.655 ppm. The concentration of the sample in 100 ML is equal to 2.655 mg/ml. The concentration of the sample in 1 ml= 26.5mg/ml.

Each 100 mg of the raw *P. oleracea* contained 26.55 mg of apigenin = 26.55%.

Hot plate test

When compared to the control, the hot plate test's outcomes indicated that the reaction time to thermal stimuli was largly longer (P≤0.01) in all treatment groups. The group treated with Apigenin recorded the highest reaction time to thermal stimuli (13.76 ± 0.55 seconds). In contrast, the group that was treated with diclofenac had the reaction time to thermal stimuli (11.48 ± 0.76 second) came in second and third degree respectively in compared with the control group, which had the lowest value (3.99 ± 0.13 second), (Table 1).

Table 1: Analgesic effect of apigenin in comparison with diclofenac using of hot plate method.

Groups of mice	Reactive time to thermal stimuli (second)
Control group (distilled water)	3.99 ±0.13 c
Treated orally with Apigenin	13.76 ±0.55 a
Treated orally with Diclofenac	11.48 ±0.76 b
LSD value	1.701 **
Significance ** (P≤0.01) N=7	

The transient pain induced by high temperatures in the hot plate test is utilized to evaluate the analgesic effect of compounds that act both centrally and peripherally on the nervous system. The previous studies (Sarmento-Neto *et al.*, 2015; Mondal *et al.*, 2022) agree with ours, which recorded that apigenin's analgesic activity is most likely mediated by central action (supra-spinally and spinally). It functions similarly to tramadol by binding to opioid receptors and generating negligible adverse effects (Pinheiro *et al.*, 2012). To determine the mechanism by which apigenin induces nociception in central ants, traditional antagonists of cholinergic and opioid receptors (atropine, mecamylamine, and naloxone, respectively) and an inhibitor of the nitric oxide pathway (L-NAME) were utilized. The findings showed that cholinergic receptors are involved, as the anti-nociceptive effects of dichloromethane fraction and apigenin were inhibited by atropine. It is known that painful stimuli increase acetylcholine in the spinal cord, which is a primary site of action for cholinomimetics in analgesia. Excitatory transmitter release is decreased, while inhibitory transmitter release is increased when muscarinic receptors in the spinal cord are activated. In this part, the cholinergic systems and opioid are utilized in mediating the anti-nociceptive influnces of apigenin, which may contribute to the observed anti-nociceptive effect.

Diclofenac Na also has an anti-nociceptive effect as a standard. It inhibits pain by interfering with prostaglandin production via competitive inhibition of cyclooxygenase, nonselective COX inhibitors, as found in its mechanism research of (Chandel *et al.*, 2018; Kaplan *et al.*, 2018).

Acetic acid test

The acetic acid test recorded that the highest value in the number of writhing per 10 min was in the control group (42.2±1.39). In comparison with other groups, there were differences but not significant between the group treated with apigenin (27.2±4.11) and diclofenac (23.8±3.36), which recorded the lowest number of writhing values (Table 2).

Table 2: Analgesic effect of apigenin in comparison with diclofenac using of acetic acid method.

Groups of mice	Number of writhing (during 10 min)
Control Group (distilled water) and Acetic acid (0.1v/v, 0.25 ml IP)	42.2 ±1.39 a
Treated orally with Apigenin and Acetic acid (0.1v/v, 0.25 ml IP)	27.2 ±4.11 b
Treated orally with Diclofenac and Acetic acid (0.1v/v, 0.25 ml IP)	23.8 ±3.36 b
LSD value	9.779 **
Significance ** (P≤0.01) N=7	

To evluate the central and peripheral influnces of analgesics in mice, the writing test employs a chemical technique involving intraperitoneal administration of acetic acid to induce pain (Bagheri *et al.*, 2015; Fattollah *et al.*, 2023). The analgesic activity indicated a reduction in frequency. Dannerman first described the manifestations of abdominal writhing in mice as an extension of the hind limbs, an arching of the back, and a contraction of abdominal muscle (Dannerman, 1977).

The analgesic effects of apigenin are almost the same as the effects of diclofenac and agree with previous studies (Sadraei *et al.*, 2017; Juárez-Chairez *et al.*, 2022). Through lipopolysaccharide influence, apigenin inhibits the induction of NO-synthase and COX2 enzymes in macrophages, thereby exerting anti-inflammatory effects. Apigenin inhibits the production of IL-4 as well. By interfering with the transcription of Nuclear Factor kappa-light-chain-Enhancer of Activated B cells (NF-κB) and conceivably by upregulating adhesion molecule in response to TNF-α, it is possible to inhibit TNF-α elevation. Apigenin exhibits similar antioxidant and

proliferative properties as other bioflavonoid compounds. It inhibits the cell cycle, enhances the activity of hepatic detoxification enzymes, and possesses a limited anti-inflammatory effect. Nevertheless, these findings agreed with other studies where the inhibitory effects of apigenin on cyclooxygenase-2 (COX-2) and monocyte adhesion to the endothelium of the umbilical vein were demonstrated through the downregulation of cellular adhesion molecules, including vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) (Toma *et al*., 2020; Shibrya *et al*., 2023).

Formalin test

The nociceptive response with the highest value (31.4±1.98) in the control group and significantly (P≤0.01) larger than in the other groups, while the group treated with diclofenac recorded the lowest value (24.2±1.15) of nociceptive response without significant deference (P≤0.01) with apigenin group when compared with it, the decreasing in the nociceptive response (26.4±0.92) in the group that treated with apigenin (Table 3).

Table 3: Analgesic effect of apigenin in comparison with diclofenac using of formalin test.

Groups of mice	Nociceptive response (number of flinching and paw licking) During 15 minutes after injection of ten microliters of 2% formalin
Control Group (distilled water)	31.40 ± 1.98 a
Treated orally with Apigenin	26.40 ± 0.92 b
Treated orally with Diclofenac	24.20 ± 1.15 b
LSD value	4.415 **
Significance ** (P≤0.01) N=7	

In mice, the formalin experiment is a realistic and trustworthy test of pain feeling and is sensitive to many kinds of pain relief drugs. The noxious stimulation consists of a 2% diluted formalin injection into the skin below the right hind skin. For 15 minutes after formalin injection, the response feedback is the number of licking and flinching of the injected paw (Hunskaar and Hole, 1987). In two distinct phases, formalin injection induced a pain-induced lapping response on the injected paw. During the initial phase, neurogenic pain results from the direct chemical stimulation of nociceptors. Inflammatory pain emanating from a combination of peripheral tissue inflammation and central sensitization mechanisms defines the second phase. Various mediators, including PGE_2, nitric oxide, neuropeptides, excitatory amino acids, and kinase inhibitors, are involved in this phase (Tjolen *et al*., 1992; Pepino *et al*., 2023).

The results agreed with Mohammad *et al*. (2017), who identified antioxidant and anti-inflammatory properties in apigenin. These have been observed in a reduction in paw edema and licking time. As a result of apigenin's inhibition of mitochondrial SDH activity, MDA levels in paw and liver tissues were reduced while SOD activity increased. Furthermore, this research has demonstrated that apigenin exhibits anti-inflammatory properties through a minor inhibition of pro-inflammatory cytokines, including IL-1β. Also, it was approved by another work that proved the anti-nociceptive impact of isolated apigenin, resulting in a diminished lapping response during both phases of the formalin model (Lopes *et al*., 2020).

Tail flick test

The test showed a large decrement in withdrawal time per second in the control group (2.83±0.34), while the group treated orally with Apigenin (11.49±1.07) and in the group treated with diclofenac (11.89±1.79), has increscent in the withdrawal time but non-significant variance in these groups (Table 4).

Table 4: Analgesic effect of apigenin in comparison with diclofenac using of tail flick method.

Groups of mice	Time of withdrawal (sec.) of tail
Control Group (distilled water)	2.83 ± 0.34 b
Treated orally with apigenin	11.49 ± 1.07 a
Treated orally with diclofenac	11.89 ± 1.79 a
LSD value	3.772 **
Significance ** (P≤0.01) N=7	

The experiment is utilized in animal investigations to assess acute pain. The heat stimulation was applied from the tail's caudal tip, and tail-flick latency was expressed as the duration that passed from the initiation of heat stimulation and the tail's motion (Brojeni *et al*., 2019). Animals' pain responses are evaluated using the tail-flick test, which is comparable to the heated plate test. It is employed in fundamental pain research and analgesic efficacy evaluation via heat reaction monitoring (D'Amour and Smith, 1941).

Our finding revealed that apigenin had an anti-nociceptive effect, as approved by earlier studies claiming that apigenin could directly affect the neural system. According to their results, the tail-flick test yielded positive results, which indicate that apigenin may exert some influence on nociceptive pathways via opioid receptors (Zarei *et al*., 2017). Different studies declared that the tail-flick response is a spinally mediated reflex, and apigenin dose-dependently increased the pain threshold (Richardson *et al*., 1998; Mondal *et al*., 2022). The latency profile of various doses of apigenin was similar to that of the standard drug tramadol. This is because receptor

stimulation is frequently linked to relief from pain and effectively controls non-analgesic effects of thermal pain via the μ receptors. These effects include physical dependence and respiratory depression. Activation of opioid subtype receptors induces spinal analgesia, while constipation is a common adverse effect. Therefore, based on the analysis of this data, it is our view that apigenin's analgesic activities are usually regulated through central action, specifically in the spine and supraspinal region. Furthermore, its binding to opioid receptors seems like that of tramadol, resulting in minimal adverse effects. Consequently, apigenin may serve as a more viable alternative to opioid drugs such as morphine when it comes to the treatment of chronic pain associated with cancer.

CONCLUSIONS AND RECOMMENDATIONS

Apigenin has an analgesic effect compared with diclofenac; however, further studies are required to deal with other medicinal uses of apigenin. Also, there is a need to study the analgesic effect of apigenin using another extraction and isolation method.

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NOVELTY STATEMENT

The study's novelty is focused on the analgesic effect of apigenin obtained from *P. oleracea* L in albino mice *in vitro*.

AUTHOR'S CONTRIBUTION

Each author made an equal contribution.

Ethical approval

The license for this work is obtained from the University of Baghdad/ College of Veterinary Medicine/ the Scientific Committee of the Department of Physiology, Biochemistry and Pharmacology located in Baghdad, Iraq.

Conflict of interest

The authors have declared no conflict of interest.

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