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Evaluation of the analgesic activity of quercetin and chrysin in animal models

Research Article

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Abstract

Pain is relieved by analgesics that acts via modulating the central and peripheral pain mediators. Some of the analgesic has been reported to have a serious adverse effects when used in long-term treatments. Therefore, there is a need of developing a potent analgesic with minimal of no adverse effects. In the current study, quercetin and chrysin were evaluated for the analgesic activity in mice and rat by hyperalgesia (Eddy's hot plate and Tail flick analgesiometer). Diclofenac and tramadol was used as a standard reference drugs. Male Swiss Albino mice and rats are divided into six groups and treated with vehicle (1% acacia), quercetin (40 mg/kg), chrysin (100 mg/kg), chrysin (200 mg/kg), diclofenac (10 mg/kg) and tramadol (10 mg/kg) orally. In the present study, diclofenac, tramadol, quercetin and chrysin significantly increased the tail flick latency in the tail flick pain model compared to control group. Chrysin showed remarkable analgesic activity at 200 mg/kg compared to 100 mg/kg in Tail Flick and Eddy's hot plate model. The current study also reported that diclofenac has more analgesic activity than tramadol on both 1st and 7th day of treatment.

Key Words: Quercetin, Chrysin, Diclofenac, Tramadol, Hyperalgesia, Analgesics, Analgesiometer.

Introduction

Analgesics affect the peripheral and central nerve systems in a variety of ways. They include paracetamol, non-steroidal anti-inflammatory medications [NSAIDs] such as the salicylates, and opioid drugs such as morphine and opium, which reversibly remove feeling (1). The degree of the pain and its reaction to other medications dictate the type of painkiller to use; the World Health Organization's [WHO] pain ladder lists moderate analgesics as the first level (2). Analgesia or pain is an unpleasant, ill-defined feeling elicited by a stimulus [external/internal] - the most significant symptom that serves as a warning signal and is largely defensive in nature. TNF, ILs provide analgesia by blocking the pain nerve sensitizing mechanism. An analgesic is a medicine that selectively reduces pain by acting in the central nervous system (CNS) or on peripheral pain mechanisms without affecting consciousness. Pain is a warning signal that is largely protective in nature, but it causes discomfort and suffering; in certain cases, it can be excruciating and impairing (3). Other symptoms of excessive pain include a sinking feeling, fear, perspiration, nausea, palpitation, a rise or dip in blood pressure, and tachypnoea. Analgesics treat pain as a symptom rather than the underlying cause. Analgesics are used to relieve pain and inflammation (4).

Primary afferent nociceptors were activated or sensitised by a number of pain-producing substances. Injured cells or moving leukocytes flowing out of arteries into the region of the cell damage release potassium, histamine, and serotonin. Clearly, transduction includes a variety of chemical reactions that work together to stimulate the main afferent nociceptor (5,6). Any of these compounds may theoretically tested to determine the peripheral stimulation for pain. In practise, practitioners do not have access to such tests. It's worth noting that most of what we know about primary afferent nociceptors comes from research on cutaneous nerves. Despite the significance of this study, mechanisms in the deep musculoskeletal or visceral tissues produce the bulk of clinically severe pain (7).

When sensory nerve endpoints in the peripheral nerves are overheated, they may feel warm, hot, or unpleasant. Place a metal probe on the skin and progressively heat up (starting at 32 degrees Celsius) until a warm-sensation and heat-pain threshold is reached. Warmth is frequently elicited at temperatures of 34 – 37 °C, whereas pain is produced around 42 – 48°C. Heat thresholds are affected by the ambient temperature, the rate of heating (1–10 C/s), the type (hairy or glabrous) and position of sample surface, the method of heat transfer, study designs, and body

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temperature. Similar to the heat threshold, the human cold threshold may be calculated. A metal probe is placed on the skin at the heat threshold, and the temperature (which commonly starts at 32 °C) lowers until the cold or pain threshold is achieved. A pleasant or innocuous experience of cold is commonly induced at around 23-29 °C, while the sensation of cold pain varies greatly and is a multimodal cold with a mode critical temperature of 23.7 °C. The analgesic limit ratio, 13.2 °C or 1.5 °C, was recently published. When chilled to at least 22 °C, nevertheless, the majority of individuals' experience cold discomfort. Many factors, including ambient temperature, cooling, and the presence of a fever, might influence these readings. Clinically distinguishing between allodynia and hyperalgesia of the common cold can be challenging due to the heterogeneity of the cold pain threshold (8).

Flavonoids are currently widely used in nutraceutical, pharmacological, medical, and cosmetic products. This is owing to their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, as well as their capacity to modify essential cellular enzyme performance (9). These are a type of natural product that is abundant in fruits, vegetables and some beverages. They are a type of phytochemical with a polyphenolic structure (10,11). The objective of this study was to evaluate the analgesic activity of selected flavonoids (quercetin and chrysin).

Materials and methods

Quercetin and chrysin procured from Sigma Aldrich, USA. Diclofenac and tramadol was procured from Life line formulations, Vijayawada. All other chemicals are analytical grade used. The study protocol was approved by Institutional Animal Ethics Committee. The protocol number was VIAEC/ 2022/7/23/07.

Evaluation of analgesic activity using Eddy's hot plate and tail immersion test

Swiss albino mice with the age 8-12 weeks and 20-25gms weight grouped into six for the Eddy's hot plate and tail immersion test in hot water. The animals were treated with following for 7 consecutive days

- Group-1: Acacia (1 %)
- Group-2: Diclofenac (10 mg/kg)
- Group-3: Tramadol (10 mg/kg)
- Group-4: Quercetin (40 mg/kg)
- Group-5: Chrysin (100 mg/kg)
- Group-6: Chrysin (200 mg/kg)

The animals are placed on the hot plate (55°C to 56 °C) and time either licking or jumping occurred was recorded before and after 20, 60 and 90 min (12). The time taken by the mice to withdraw the tail from hot water (55 ± 1°C.) was noted as reaction time. The cut off time was considered 10-12 sec (13).

Evaluation of analgesic activity using Tail flick analgesiometer

Albino rats (150-180 g) were grouped into six and were treated with following for 7 consecutive days

- Group-1: Acacia (1 %)
- Group-2: Diclofenac (10 mg/kg)
- Group-3: Tramadol (10 mg/kg)
- Group-4: Quercetin (40 mg/kg)
- Group-5: Chrysin (100 mg/kg)
- Group-6: Chrysin (200 mg/kg)

The tail flick model is the second most extensively used animal model for assessing analgesic efficacy in rats or mice. When a mouse's tail comes into touch with heat or thermal stimulation, it will try to withdraw or flick his tail away from the source of the stimuli. It represents the average reaction time for pain perception and is used as a benchmark. This is a behavior that may be applied to humans as well. After the rat's tail is flicked, they are given analgesic medicine and their reaction time is measured again. There will be a delay in reaction time if the medicine has analgesic properties. Rats respond in 3 to 5 seconds on average; if it takes more than 10-12 seconds, the rats will be removed from the experiment to avoid additional harm (12).

Results

Heat–induced hyperalgesia using Eddy's hot plate

Table 1 shows that diclofenac, tramadol, quercetin and chrysin were significantly increased the reaction time to heat-induced hyperalgesia by 183 %, 138 %, 134 %, 121% at 100 mg/kg and 133% at 200 mg/kg, respectively compared to control after 30 min of treatment on 1st day. The same drugs were significantly increased the reaction time to heat-induced hyperalgesia by 171 %, 160 %, 154 %, 138 % at 100 mg/kg and 150% at 200 mg/kg, respectively compared to control after 60 min of treatment on 1st day also. Similarly, the selected drugs and flavonoids significantly increased the reaction time to heat-induced hyperalgesia by 164%, 154%, 147%, 144% at 100 mg/kg and 137% at 200 mg/kg, respectively compared to control after 90 min of treatment on 1st day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the reaction time to heat-induced hyperalgesia by 190 %, 170 %, 175 %, 168 % at 100 mg/kg and 155 % at 200 mg/kg, respectively compared to control after 30 min of treatment on 7th day.

Likewise, all the drugs enhanced the reaction time to heat-induced hyperalgesia by 178 %, 168 %, 172%, 161 % at 100 mg/kg and 162 % at 200 mg/kg, respectively compared to control after 60 min of treatment on 7th day. Similarly, the selected drugs were significantly increased the reaction time to heat-induced hyperalgesia by 191 %, 178 %, 176 %, 171 % at 100 mg/kg and 176 % at 200 mg/kg, respectively compared to control after 90 min of treatment on 7th day. Diclofenac significantly increased the reaction time to heat-induced hyperalgesia after treatment (214 %) at 30 minutes, (198 %) at 60 minutes, and (198 %) at 90 minutes on the first day, and (220 %) at 30 minutes, (218 %) at 60 minutes, and (227 %) at 90 minutes on the seventh day. Tramadol was significantly increased the reaction time to heat-induced hyperalgesia after treatment (164 %) at 30 minutes, (191 %) at 60 minutes and (190 %) at 90 minutes on 1st day and (200 %) at 30 minutes, (210 %) at 60 minutes, (216%) at 90 minutes

on 7th day. Quercetin was significantly increased the reaction time to heat-induced hyperalgesia after treatment (152 %) at 30 minutes, (173 %) at 60 minutes and (176 %) at 90 minutes on 1st day and (196 %) at 30 minutes, (205 %) at 60 minutes, (203 %) at 90 minutes on 7th day.

Chrysin at 100mg/kg was significantly increased the reaction time to heat-induced hyperalgesia after treatment (153 %) at 30 minutes, (186 %) at 60 minutes and (182 %) at 90 minutes on 1st day, and (204 %) at 30 minutes, (208 %) at 60 minutes, (214 %) at 90 minutes on 7th day. Chrysin at 200 mg/kg was significantly increased the reaction time to heat-induced hyperalgesia after treatment (172 %) at 30 minutes, (191 %) at 60 minutes and (183 %) at 90 minutes on 1st day and (198 %) at 30 minutes, (220 %) at 60 minutes, (231 %) at 90 minutes on 7th day. Diclofenac and tramadol significantly increased the reaction time to heat-induced hyperalgesia by 183 % and 138 % respectively compared to control after 30 min of treatment on 1st day. On the 7th day, the reaction time to heat-induced hyperalgesia was significantly increased by 191% and 170 % with diclofenac and tramadol respectively.

The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment. Diclofenac and tramadol was significantly increased the reaction time to heat-induced hyperalgesia by 171 % and 162 % respectively compared to control after 60 min of treatment on 1st day. On 7th day, the reaction time to heat-induced hyperalgesia was significantly increased by 178 % and 168 % with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment. Diclofenac and tramadol was significantly increased the reaction time to heat-induced hyperalgesia by 171 % and 162 % respectively compared to control after 90 min of treatment on 1st day. On 7th day, the reaction time to heat-induced hyperalgesia was significantly increased by 191 % and 178 % with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment.

Table 1: Effect of diclofenac, tramadol, quercetin and chrysin on the reaction time (sec) of mice to heat – induced hyperalgesia using Eddy's hot plate

Treatment	Before Treatment	After Treatment					
		1st day			7th day		
		30 min	60 min	90 min	30 min	60 min	90 min
Control	4.683 ± 0.765	4.817 ± 0.387	4.783 ± 0.475	4.983 ± 0.313	4.767 ± 0.266	5.067 ± 0.615	4.900 ± 0.341
Diclofenac (10 mg/ kg)	4.133 ± 0.589	8.850 ± 1.154 a,b	8.217 ± 1.646 a,b	8.217 ± 0.939 a,b	9.100 ± 0.867 a,b	9.033 ± 0.463 a,b	9.383 ± 0.821 a,b
Tramadol (10 mg/ kg)	4.050 ± 0.505	6.650 ± 0.922 a,b	7.750 ± 0.764 a,b	7.700 ± 0.894 a,b	8.117 ± 0.624 a,b	8.533 ± 0.819 a,b	8.767 ± 0.766 a,b
Quercetin (40 mg/ kg)	4.250 ± 0.373	6.483 ± 0.768 a,b	7.367 ± 0.413 a,b	7.483 ± 0.993 a,b	8.367 ± 0.720 a,b	8.717 ± 0.714 a,b	8.633 ± 0.635 a,b
Chrysin (100 mg/kg)	3.933 ± 0.468	5.867 ± 0.489 a,b	6.633 ± 0.344 a,b	7.183 ± 0.232 a,b	8.033 ± 0.314 a,b	8.183 ± 0.412 a,b	8.417 ± 0.343 a,b
Chrysin (200 mg/kg)	3.733 ± 0.539	6.450 ± 0.521 a,b	7.183 ± 0.354 a,b	6.850 ± 0.476 a,b	7.400 ± 0.297 a,b	8.233 ± 0.320 a,b	8.633 ± 0.418 a,b

[a]$P < 0.05$ compared to control; [b]$P < 0.05$ compared to before treatment

Heat – induced hyperalgesia using tail immersion method

Table 2 shows that in tail immersion induced method, diclofenac, tramadol, quercetin and chrysin were significantly increased the reaction time to tail withdrawal reflexes of mice by 163 %, 199 %, 215 %, 196 % at 100 mg/kg and 209 % at 200 mg/kg, respectively compared to control after 30 min of treatment on 1st day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the reaction time to tail withdrawal reflexes of mice, as shown in Table 14 by 152 %, 227 %, 228 %, 218 % at 100 mg/kg and 226 % at 200 mg/kg, respectively compared to control after 60 min of treatment on 1st day. The selected drugs and compounds were significantly increased the reaction time to tail withdrawal reflexes of mice by 146 %, 219 %, 222 %, 216 % at 100 mg/kg and 226 % at 200 mg/kg, respectively compared to control after 90 min of treatment on 1st day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the reaction time to tail withdrawal reflexes of mice by 193 %, 217 %, 260 %, 184 % at 100 mg/kg and 132 % at 200 mg/kg, respectively compared to control after 30 min of treatment on 7th day. Similarly, the drugs and compounds significantly increased the reaction time to tail withdrawal reflexes of mice by 189 %, 218 %, 267 %, 173 % at 100 mg/kg and 137 % at 200 mg/kg, respectively compared to control after 60 min of treatment on 7th day.

Diclofenac, tramadol, quercetin and chrysin were significantly increased the reaction time to tail withdrawal reflexes of mice by 188 %, 205 %, 274 %, 183 % at 100 mg/kg and 140 % at 200 mg/kg, respectively compared to control after 90 min of

treatment on 7th day. Diclofenac was significantly increased the reaction time to tail withdrawal reflexes of mice after treatment (181 %) at 30 minutes, (157 %) at 60 minutes and (157 %) at 90 minutes on 1st day and (203 %) at 30 minutes, (199 %) at 60 minutes, (190 %) at 90 minutes on 7th day. Tramadol was significantly increased the reaction time to tail withdrawal reflexes of mice after treatment (241 %) at 30 minutes, (257 %) at 60 minutes and (258 %) at 90 minutes on 1st day and (251 %) at 30 minutes, (251 %) at 60 minutes, (227 %) at 90 minutes on 7th day. In tail immersion induced method, quercetin was significantly increased the reaction time to tail withdrawal reflexes of mice after treatment (164 %) at 30 minutes, (162 %) at 60 minutes and (164 %) at 90 minutes on 1st day and (188 %) at 30 minutes, (194 %) at 60 minutes, (191 %) at 90 minutes on 7th day.

Chrysin at 100mg/kg was significantly increased the reaction time to tail withdrawal reflexes of mice after treatment (198 %) at 30 minutes, (205 %) at 60 minutes and (212 %) at 90 minutes on 1st day and (177 %) at 30 minutes, (166 %) at 60 minutes, (169 %) at 90 minutes on 7th day. Chrysin 200 mg/kg was significantly increased the reaction time to tail withdrawal reflexes of mice after treatment (279 %) at 30 minutes, (280 %) at 60 minutes and (293 %) at 90 minutes on 1st day and (167 %) at 30 minutes, (174 %) at 60 minutes, (171 %) at 90 minutes on 7th day. Diclofenac and tramadol was significantly increased to tail withdrawal reflexes of mice by 163 % and 199 % respectively compared to control after 30 min of treatment on 1st day. On 7th day, the reaction time to tail withdrawal reflexes of mice was significantly increased by 193 % and 217 % with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment.

Diclofenac and tramadol was significantly increased to tail withdrawal reflexes of mice by 152 % and 227 % respectively compared to control after 60 min of treatment on 1st day. On 7th day, the reaction time to tail withdrawal reflexes of mice was significantly increased by 187 % and 216 % with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment. Diclofenac and tramadol was significantly increased to tail withdrawal reflexes of mice by 146 % and 219 % respectively compared to control after 90 min of treatment on 1st day. On 7th day, the reaction time to tail withdrawal reflexes of mice was significantly increased by 188% and 205 % with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment.

Table 2: Effect of diclofenac, tramadol, quercetin and chrysin on the reaction time (sec) of mice to heat – induced hyperalgesia using tail immersion method

Treatment	Before Treatment	After Treatment					
		1st day			7th day		
		30 min	60 min	90 min	30 min	60 min	90 min
Control	3.518 ± 0.818	3.487 ± 0.754	3.255 ± 0.758	3.395 ± 0.617	3.320 ± 0.418	3.347 ± 0.599	3.192 ± 0.212
Diclofenac (10 mg/ kg)	3.150 ± 0.719	5.718 ± 0.625^{a,b}	4.975 ± 0.664^{a,b}	4.958 ± 1.058^{a,b}	6.412 ± 0.576^{a,b}	6.292 ± 1.199^{a,b}	6.013 ± 0.948^{a,b}
Tramadol (10 mg/ kg)	2.880 ± 0.651	6.952 ± 1.763^{a,b}	7.412 ± 1.467^{a,b}	7.452 ± 1.554^{a,b}	7.230 ± 1.081^{a,b}	7.247 ± 0.671^{a,b}	6.560 ± 0.436^{a,b}
Quercetin (40 mg/ kg)	4.575 ± 0.531	7.518 ± 0.631^{a,b}	7.442 ± 1.353^{a,b}	7.537 ± 1.255^{a,b}	8.637 ± 0.728^{a,b}	8.877 ± 0.721^{a,b}	8.747 ± 0.734^{a,b}
Chrysin (100 mg/kg)	3.460 ± 0.556	6.857 ± 1.481^{a,b}	7.102 ± 1.564^{a,b}	7.367 ± 1.790^{a,b}	6.138 ± 0.823^{a,b}	5.757 ± 1.537^{a,b}	5.862 ± 1.558^{a,b}
Chrysin (100 mg/kg)	2.623 ± 0.554	7.320 ± 1.557^{a,b}	7.362 ± 1.462^{a,b}	7.698 ± 0.766^{a,b}	4.403 ± 0.728^{a,b}	4.567 ± 0.902^{a,b}	4.498 ± 0.58^{a,b}

$^{a}P < 0.05$ compared to control; $^{b}P < 0.05$ compared to before treat

Tail flick latency in tail flick model of pain in rats

Table 3 showed that diclofenac, tramadol, quercetin and chrysin were significantly increased the tail flick latency in tail flick pain model using rats by 232 %, 174 %, 165 %, 182 % at 100 mg/kg and 170 % at 200 mg/kg, respectively compared to control after 30 min of treatment on 1st day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the tail flick latency in tail flick pain model using rats as shown in Table 21 by 236 %, 180 %, 174 %, 207 % at 100 mg/kg and 178 % at 200 mg/kg, respectively compared to control after 60 min of treatment on 1st day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the tail flick latency in tail flick pain model using rats by 266 %, 182 %, 190 %, 225 % at 100 mg/kg and 195 % at 200 mg/kg, respectively compared to control after 90 min of treatment on 1st day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the tail flick latency in tail flick pain model using rats by 221 %, 140 %, 173 %, 156 % at 100 mg/kg and 162 % at 200 mg/kg, respectively compared to control after 30 min of treatment on 7th day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the tail flick latency in tail flick

pain model using rats by 232 %, 244 %, 178 %, 166 % at 100 mg/kg and 180 % at 200 mg/kg, respectively compared to control after 60 min of treatment on 7th day. Diclofenac, tramadol, quercetin and chrysin were significantly increased the tail flick latency in tail flick pain model using rats by 254 %, 165 %, 200 %, 172 % at 100 mg/kg and 194 % at 200 mg/kg, respectively compared to control after 90 min of treatment on 7th day.

Diclofenac was significantly increased the tail flick latency in tail flick pain model using rats after treatment (366 %) at 30 minutes, (359 %) at 60 minutes and (384 %) at 90 minutes on 1st day and (356 %) at 30 minutes, (363 %) at 60 minutes, (379 %) at 90 minutes on 7th day. Tramadol was significantly increased the tail flick latency in tail flick pain model using rats after treatment (258 %) at 30 minutes, (255 %) at 60 minutes and (264 %) at 90 minutes on 1st day and (211 %) at 30 minutes, (216 %) at 60 minutes, (231 %) at 90 minutes on 7th day. Quercetin was significantly increased the tail flick latency in tail flick pain model using rats after treatment (194 %) at 30 minutes, (160 %) at 60 minutes and (166 %) at 90 minutes on 1st day and (169 %) at 30 minutes, (173 %) at 60 minutes, (182 %) at 90 minutes on 7th day. Chrysin at 100 mg/kg was significantly increased the tail flick latency in tail flick pain model using rats after treatment (258 %) at 30 minutes, (255 %) at 60 minutes and (264 %) at 90 minutes on 1st day and (211 %) at 30 minutes, (216 %) at 60 minutes, (231 %) at 90 minutes on 7th day. Chrysin at 200mg/kg was significantly increased the tail flick latency in tail flick pain model using rats after treatment (170 %) at 30 minutes, (171 %) at 60 minutes and (260 %) at 90 minutes on 1st day and (186 %) at 30 minutes, (187 %) at 60 minutes, (184 %) at 90 minutes on 7th day.

Diclofenac and tramadol was significantly increased the tail flick latency in tail flick pain model using rats by 232 % and 174 % respectively compared to control after 30 min of treatment on 1st day. On 7th day, the tail flick latency in tail flick pain model using rats was significantly increased by 221 % and 140 % with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment. Diclofenac and tramadol was significantly increased the tail flick latency in tail flick pain model using rats by 236 % and 180 % respectively compared to control after 60 min of treatment on 1st day. On 7th day, the tail flick latency in tail flick pain model using rats was significantly increased by 232% and 147% with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment. Diclofenac and tramadol was significantly increased the tail flick latency in tail flick pain model using rats by 266 % and 182 % respectively compared to control after 90 min of treatment on 1st day. On 7th day, the tail flick latency in tail flick pain model using rats was significantly increased by 254 % and 165 % with diclofenac and tramadol respectively. The present study results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment.

Table 3: Tail flick latency with chrysin (200 mg/kg) in tail flick model of pain in rats

Treatment	Before Treatment	After Treatment					
		1st day			7th day		
		30 min	60 min	90 min	30 min	60 min	90 min
Control	5.183 ± 1.556	5.917 ± 1.651	5.683 ± 1.480	5.400 ± 2.023	6.033 ± 1.929	5.850 ± 1.567	5.600 ± 1.503
Diclofenac (10 mg/kg)	3.750 ± 1.636	13.733 ± 2.104[a,b]	13.467 ± 1.027 [a,b]	14.400 ± 1.587 [a,b]	13.38 ± 1.245 [a,b]	13.62 ± 1.172 [a,b]	14.23 ± 1.061 [a,b]
Tramadol (10 mg/kg)	4.000 ± 0.837	10.350 ± 1.415 [a,b]	10.233 ± 1.015 [a,b]	9.867 ± 1.211 [a,b]	8.450 ± 2.071 [a,b]	8.650 ± 1.535 [a,b]	9.267 ± 1.601 [a,b]
Quercetin (40 mg/kg)	6.167 ± 1.506	9.250 ± 1.720 [a,b]	9.900 ± 1.837 [a,b]	10.267 ± 1.822 [a,b]	10.47 ± 1.035 [a,b]	10.70 ± 1.260 [a,b]	11.25 ± 1.307 [a,b]
Chrysin (100 mg/kg)	4.667 ± 0.986	10.783 ± 1.777 [a,b]	11.883 ± 1.451 [a,b]	12.167 ± 1.680 [a,b]	9.417 ± 2.972 [a,b]	9.733 ± 2.748 [a,b]	9.983 ± 2.541 [a,b]
Chrysin (200 mg/kg)	5.917 ± 1.463	10.083 ± 1.109 [a,b]	10.150 ± 0.715 [a,b]	10.517 ± 1.262 [a,b]	9.967 ± 1.904 [a,b]	10.58 ± 1.472 [a,b]	10.90 ± 1.479 [a,b]

[a]$P < 0.05$ compared to control; [b]$P < 0.05$ compared to before treatment

Discussion

The major goal of this work is to evaluate the analgesic activity of selected flavonoids chrysin and quercetin in rats and mice. The results proven that the selected flavonoids have analgesic effect using hot plate analgesiometer and tail-flick methods. The present study results are consistent with the previous study reports performed with flavonoids. Previous investigations have shown that luteolin (1 mg/kg, oral) could also exhibits analgesic activity which is reported using hot plate method which could increase the pain threshold of hot-plate model and diminish the incidence of writhing model with inhibition of 30 % and 25 %, respectively (14).

Similarly, the researcher reported that hesperidin showed antinociceptive activities in the acetic acid-induced writhing test (0.6 mg/kg and 1 mg/kg, $p<0.01$)

and hotplate test (10mg/kg, $p<0.01$ and 30 mg/kg, $p<0.05$), and the activity was observed only when intraperitoneal administered, while its aglycone-hesperetin did not exhibit any effect in vivo in mice. In this work we have reported that the selected flavonoids chrysin and qurecetin are having analgesic effect at the dosage of (100 mg/kg, 200 mg/kg) for chrysin and (40 mg/kg) for qurecetin using hot plate method which significantly increased the pain in mice and rats when we compared the selected flavonoids with control group we have got $^{a}P < 0.05$ compared to control the mean value is The percentage was exhibited (134 percent) for the selected flavonoids qurecetin at a dose of (40 mg/kg) at 30 minutes in the 1st day, chrysin 100mg At 30 minutes, the percentage was shown to be (121 %), and at 30 minutes, the percentage was revealed to be (133 %) on the first day of therapy.

The antinociceptive activity of mice was studied using the tail-flick method, according to the researcher. To evaluate response latencies, a radiant heat automated tail-flick analgesiometer was used. By placing the tip (final 1-2 cm) of the tail on a radiant heat source, the basal response time of animals to radiant heat was measured. The endpoint was the separation of the tail from the radiant warmth. Heat-related tail injury was avoided by using a 15-second cutoff time. The mice were separated into five groups and given morphine (10 mg/kg), normal saline, and leonurus cardiaca (125, 250, and 500 mg/kg) treatments. At 30, 45, 60, 75, and 90 minutes following the injection of medicines, the latent time of the tail-flick reaction was assessed. *Caesalpinia bonducella (L.) Fleming* flower extract (CBFE) was given orally (30, 100, and 300 mg/kg) and examined for analgesic effects. The mice were chosen 24 hours before the test based on their response in the model, with those mice that remained on the apparatus for more than 10 seconds being eliminated. Complete analgesia was defined as a 20-second cutoff time. Control mice and animals prepared with a test chemical or morphine had their response times measured (15).

The present study also reported that the tail-flick method was used to evaluate the analgesic activity in rats. To evaluate response latencies, a radiant heat automated tail-flick analgesiometer was used. By placing the tip (final 1-2 cm) of the tail on a radiant heat source, the basal response time of animals to radiant heat was measured. The endpoint was the separation of the tail from the radiant warmth. Heat-related tail injury was avoided by using 10-12 seconds cut off time. Rats were divided into six groups were treated with diclofenac and tramadol at the dosage (10 mg/kg, 10mg/kg) The latent period of the tail-flick response was determined at 30, 60, and 90 minutes after the administration of drugs.

The tail immersion method is a method to evaluate central analgesic activity. The lower 5 cm section of tail of mice was immersed in a beaker in which temperature of water was maintained at 55 ± 0.5°C. The time, in seconds, for tail withdrawal from the water was recorded as the reaction time; with a cut-off time for immersion set at 10 seconds. Results of tail immersion test in our study demonstrated that administration of to the mice resulted in a significantly prolonged tail withdrawal reflex time in response to heat stimuli. Kaempferol 3-*o*-sophoroside possesses significant analgesic activity in the tail clip, tail flick, tail immersion and acetic acid-induced writhing models(i.P. 50 mg/kg,). Kaempferol 3-*o*-[α-l-rhamnopyranosyl (1→6)-β-d-glucopyranoside] (3) chrysin and quercetin have showed prominent analgesic effect in both (100,200 mg/kg) and (40 mg/kg) doses with 196 % and 218 % at 30 mins in the 1st day and for quercetin 215 % at 30 minutes.

The analgesic activity of quercetin in the soleus muscle was characterized by reduction of significant acute swimming-induced NFB activation and promotion of the Nrf2/HO-1 signal transduction pathway (16). Quercetin reduced acute swimming-induced cell mobilization (MPO and NAG activity), signal transduction (TNF-, IL-1, and IL-10), inflammatory processes, COX-2 and gp91phox mRNA expression, and tissue damage in the soleus muscle, that were associated with NFB and Nrf2 regulation (CK blood concentration and MyoD mRNA expression). Furthermore, quercetin reduced severe acute swimming-induced neuro-inflammation in the spinal cord, as evaluated by lowered cytokine production, oxidative alterations, and glial cell stimulation. Quercetin also reduced MPO and NAG activity in the soleus muscle. These results are consistent with the reduction in cytokine production (TNF- and IL-1) in the soleus muscle, as well as TNF- and IL-1's chemoattractive action on neutrophils. TNF-, for example, activates neutrophils as well as macrophages.

Chrysin therapy reduced licking duration in both phases of the formalin test, as well as noradrenalin and corticosterone levels in male rats in a dose-dependent way, and it has been demonstrated that chrysin has inhibitory effects on corticosterone and catecholamine levels (16). Noradrenaline helps to reduce pain by activating the pain inhibitory system. The principal sources of noradrenaline are brain stem nuclei, locus coeruleus in the central nervous system, and sympathetic nerves in the peripheral nervous system. Through modulation of corticosterone and noradrenaline, chrysin may be beneficial in suppressing pain in both phases of the formalin test.

Conclusion

The present study has proven that the selected flavonoids chrysin and quercetin are showing analgesic activity in rats and mice using hot plate analgesiometer and tail flick method. Diclofenac, tramadol, quercetin and chrysin were significantly increased the tail flick latency in tail flick pain model using rats by 183 %, 138 %, 134 %, 121 % at 100 mg/kg and 133 % at 200 mg/kg, respectively compared to control after 30 min of treatment on. Diclofenac and tramadol was significantly increased the reaction time to heat-induced hyperalgesia by 183 % and 138 % respectively compared to control after 30 min of treatment on 1st day. On 7th day, the reaction time to heat-induced hyperalgesia was significantly increased by 191 % and 170 % with diclofenac and tramadol respectively. The present study

results indicated that diclofenac has more analgesic activity compared to tramadol on both 1st and 7th day of treatment 1st day.

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Conflicts of interest

None.

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