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Evaluation of skeletal muscle relaxant activity of quercetin and chrysin in Albino rats using Rotarod apparatus and actophotometer

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ABSTRACT

Quercetin is a natural flavonoid found abundantly in vegetables and fruits. Chrysin (5, 7-dihydroxyflavone), a natural polyphenol, occurs in many plants, honey, and propolis. Quercetin and chrysin have a blend of many pharmacological activities such as anticarcinogenic, pro-apoptotic, antiangiogenic, antimetastatic, immunomodulatory, and antioxidant properties. But there is no scientific evidence regarding the muscle relaxant activity and locomotor activities of selected flavonoids. The present study was planned to evaluate the influence of quercetin and chrysin on muscle relaxant and locomotor activities using experimental animal models. Quercetin and chrysin (20, 40 and 60 mg/kg) was administered to rats and evaluated for muscle relaxant activity using rota-rod apparatus and locomotor activity using actophotometer. The time spent on the rota rod was significantly reduced by chrysin at 20, 40 and 60 mg/kg when compared to saline (control). Quercetin also reduced the time spent but statistically not significant. The positive control, diazepam was found to be more significant ($p < 0.001$) than the test doses of chrysin. The results of the locomotor activity study indicated that chrysin significantly reduced the locomotion in rats, but quercetin has no significant activity. The results of the present study revealed the chrysin has significant ($p < 0.001$) and dose dependent muscle relaxant and locomotor depressant activities. Quercetin also reduced the muscle relaxant and locomotor activities but statistically not significant.

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Keywords
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INTRODUCTION

Skeletal muscle relaxants are a heterogeneous group of medications commonly used to treat two different types of underlying conditions: spasticity from upper motor neuron syndromes and muscular pain or spasms from peripheral musculoskeletal conditions [1]. Common musculoskeletal conditions causing tenderness and muscle spasms include fibromyalgia, tension headaches, myofascial pain syndrome, and mechanical low back or neck pain. Skeletal muscle relaxants are one of several classes of medications frequently used to treat these conditions [2-4].

Quercetin, a flavonol has been shown to possess a wide spectrum of pharmacological effects including antioxidant, antinociceptive, anti-inflammatory, neuroprotective, anticancer [5], hepatoprotective [6] and nephroprotective activities [7]. Chrysin (5, 7-

Table 1. Effect of quercetin and chrysin on muscle relaxant activity in rats

Treatment	*Fall off time (Mean ± SD)*				*Percent reduction in fall off time*		
	Before treatment	After 30 min	After 60 min	After 90 min	After 30 min	After 60 min	After 90 min
Control	191.17 ± 9.30	181.00 ± 6.90	173.17 ± 7.99	172.33 ± 8.07	5.319	9.415	9.855
Diazepam (10 mg/kg)	169.00 ± 7.72	85.00 ± 8.53[a, b]	38.33 ± 8.19[a, b]	24.00 ± 8.81[a, b]	49.704	77.319	85.798
Quercetin (20 mg/kg)	175.50 ± 7.97	169.00 ± 7.72	154.67 ± 8.57[a, b]	139.67 ± 8.07[a, b]	3.703	11.868	20.415
Quercetin (40 mg/kg)	182.17 ± 8.42	170.00 ± 8.53[a, b]	181.67 ± 6.80[a, b]	163.50 ± 7.82[a, b]	6.680	0.274	10.248
Quercetin (60 mg/kg)	164.00 ± 7.72	154.50 ± 8.41[a, b]	147.50 ± 7.97[a, b]	150.83 ± 7.99[a, b]	5.792	10.060	8.030
Chrysin (20 mg/kg)	169.17 ± 7.99	108.83 ± 8.98[a, b]	62.67 ± 8.64[a, b]	43.83 ± 8.33[a, b]	35.668	62.954	74.091
Chrysin (40 mg/kg)	178.00 ± 7.16	95.00 ± 7.54[a, b]	53.17 ± 8.61[a, b]	32.67 ± 7.45[a, b]	46.629	70.129	81.646
Chrysin (60 mg/kg)	193.83 ± 6.62	81.33 ± 8.66[a, b]	48.83 ± 8.70[a, b]	31.00 ± 7.72[a, b]	58.040	74.80	84.006

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

Table 2. Effect of quercetin and chrysin on locomotor activity in rats

Treatment	*Actophotometer scores (Mean ± SD)*				*Percent reduction in motor activity*		
	Before treatment	After 30 min	After 60 min	After 90 min	After 30 min	After 60 min	After 90 min
Control	213.17 ± 8.93	188.67 ± 7.37	170.50 ± 7.50	162.67 ± 7.37	11.52	20.01	23.69
Diazepam	177.83 ± 7.73	52.50 ± 7.50[a, b]	25.83 ± 7.39[a, b]	18.00 ± 7.16[a, b]	70.47	85.47	89.87
Quercetin (20 mg/kg)	183.67 ± 7.79	175.67 ± 7.00	161.83 ± 7.88	148.50 ± 6.57	4.30	11.88	19.14
Quercetin (40 mg/kg)	168.00 ± 6.72	149.33 ± 7.55[a, b]	148.33 ± 7.23[a, b]	143.17 ± 7.33[a, b]	11.11	11.70	14.82
Quercetin (60 mg/kg)	191.67 ± 8.21	180.50 ± 7.50[a, b]	175.33 ± 7.09[a, b]	173.00 ± 10.83[a,b]	5.82	8.50	9.70
Chrysin (20 mg/kg)	151.00 ± 7.16	87.50 ± 7.01[a, b]	29.83 ± 7.33[a, b]	18.17 ± 7.11[a, b]	42.05	80.24	88.01
Chrysin (40 mg/kg)	128.33 ± 6.65	80.67 ± 7.50[a, b]	39.33 ± 7.03[a, b]	21.67 ± 7.37[a b]	37.14	69.35	83.12
Chrysin (60 mg/kg)	142.83 ± 7.60	61.67 ± 7.37[a, b]	27.50 ± 7.42[a, b]	16.67 ± 7.09[a, b]	56.82	80.7	88.33

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

dihydroxyflavone) is a naturally occurring flavonoid that exhibits many pharmacological effects, including antioxidant [8], anticancer, hepatoprotective [9] and nephroprotective [10]. Several studies reported that chrysin has anti-inflammatory effect by inhibiting several cytokines, nitric oxide, prostaglandin E and COX-2 [11].

Till date, there is no data available regarding the skeletal muscle relaxant activity of quercetin and chrysin. Therefore, the present study was planned to investigate whether the quercetin and chrysin reduced the skeletal muscle relaxant and locomotor activities in rat models.

MATERIALS AND METHODS

Drugs and chemicals

Quercetin and chrysin were purchased from Sigma Chemical Co. (St. Louis, MO). Albendazole and diazepam was obtained as gift sample from Lifeline Formulations Pvt. Limited, India and Lupin Laboratories Ltd., India, respectively. Sodium carboxymethyl cellulose (SCMC) was purchased from Finar chemicals Ltd., Ahmadabad, India. Distilled water, prepared from deionized water, was used throughout the study. All other chemicals and reagents used were of analytical grade.

Experimental animals

Animal experiments were performed according to the institutional guidelines for the care and use of laboratory animals, and approved by the animal ethics committee of KVSR Siddhartha College of Pharmaceutical Sciences (SCOPS), Vijayawada, Andhra Pradesh, India (993/a/06/CPCSEA). Male Wistar rats (180–220 g) were procured from National Institute of Nutrition (NIN), Hyderabad, Andhra Pradesh, India. Animals were housed six per cage and given free access to food (Hindustan Lever, Mumbai, India) and water *ad libitum* in animal house at the KVSR SCOPS. Before starting the experiments, animals were kept under standard laboratory conditions (12/12 h light/darkness, 22 ± 2ºC and 50-60% humidity) for at least a week.

Evaluation of skeletal muscle relaxant activity (motor coordination)

The animals are placed on Rotarod (Dolphin Scientific Equipment, Mumbai, India) for 5 min or more after successive trials as per the method described by Chandrashekaran et al., 2013 with minor modifications [12]. The animals were divided into eight groups of six rats each. The drugs were administered as shown below:

- Group I – Control rats (normal saline 10 mL/kg)
- Group II – Standard (diazepam 10 mg/kg)
- Group III – Quercetin 20 mg/kg
- Group IV – Quercetin 40 mg/kg
- Group V – Quercetin 60 mg/kg
- Group VI – Chrysin 20 mg/kg
- Group VII – Chrysin 40 mg/kg
- Group VIII – Chrysin 60 mg/kg

After the administration of control, standard, quercetin and chrysin, the fall off time from the rotating rod was noted after 30 min. The difference in the fall off time from the rotating rod between the control and the treated rats was taken as an index of muscle relaxation.

Evaluation of Locomotor Activity

The spontaneous locomotor activity was assessed with the help of a photoactometer as described by Idris et al.,

2015 with minor modifications [13]. Each animal was observed for a period of 5 min in a square closed field arena (30 cm × 30 cm × 30 cm) equipped with six photocells in the outer wall. Interruptions of photocell beams (locomotor activity) were recorded by means of a six digits' counter. To see the locomotor activity, the actophotometer (MKM, Chennai, India) was turned on and each rat was placed individually in the activity cage for 5 min. The basal activity score for all the animals was noted. After the administration of control, standard, quercetin and chrysin orally, the activity score for 5 min was observed. The difference in the activity, before and after drug administration, was noted. The percentage decrease in motor activity was calculated.

Statistical Analysis

All statistics were calculated using Graph Pad Prism 5.0 software (San Diego, CA). The results were expressed as a mean±standard deviation. Statistical analysis was carried out by using the analysis of variance followed by Tukey's post-hoc test. The p value less than 0.05 were considered significant.

RESULTS

Rotarod Test

For muscle relaxation, chrysin showed highly significant reduction in the time spent by the animals on the revolving rod when compared to the control ($p< 0.000$). The results are summarized in Table 1. The standard drug (diazepam) also showed a highly significant effect when compared to the control ($p< 0.000$). However, three different doses of chrysin (20, 40 and 60 mg/kg) showed a dose-dependent increase in muscle relaxation, that is, 193.83±6.62 and 31.00±7.72, respectively, when compared to the control after 90 minutes of treatment. Maximum muscle relaxation was observed with 60 mg/kg of chrysin. The result from the Rotarod test showed that the chrysin significantly reduced the motor coordination of the tested animals. Quercetin also reduced the time spent on revolving rod but statistically not significant.

Actophotometer

In locomotor activity study, it was found that chrysin significantly ($P < 0.001$) depressed the locomotor activity in a dose and time dependent manner. The activities increased as time approached to 90 min. The results are summarized in Table 2. The percentage of reduction in the locomotor activity with diazepam (10 mg/kg, p. o.,) after 90 min was 89.87, that is, there was a highly significant ($P< 0.000$) decrease in locomotor activity compared to the control. Maximum muscle relaxation was observed with 60 mg/kg of chrysin. There was no statistically significant decrease in the locomotor activity with three different doses of quercetin (20, 40 and 60 mg/kg, p. o.).

DISCUSSION

In recent years, public and scientific interest in plant flavonoids has tremendously increased because of their postulated health benefits. Flavonoids are ubiquitous plant specialized metabolites that contain large groups of low-molecular-weight polyphenolic compounds, which present benefits to human health because of their biological properties. To date, approximately 5000 diverse flavonoids have been identified [14]. Nutritionists calculate the approximate average ingestion of flavonoids by humans on a normal diet to be 1-2 g/day [15]. Flavonoids are naturally occurring polyphenols with patterns of hydroxylation and substitutions that give rise to various subclasses including flavanones, anthocyanidins, flavonols, flavones, catechins (or flavanols), isoflavones, dihydroflavonols, and chalcones [16, 17].

A number of in vitro and in vivo studies have revealed the therapeutic effects of chrysin against various diseases. In general, chrysin exhibits many biological activities and pharmacological effects, including antioxidant, anti-inflammatory, anticancer, neuroprotective, colonprotective, nephroprotective, antidiabetic, hypolipidemic, antiarthritic, antiasthmatic, antidepressant, hepatoprotective, cardioprotective, and antiviral activities [18]. Flavonoids are promising skeletal muscle relaxant agents. The present study showed that the chrysin possessed muscle relaxant and locomotor depressant activities in experimental models. Previous studies concluded that the methanolic extract of *Basella Alba* possess significant antidepressant like effect and skeletal muscle relaxant activity. The activity may be due to the alkaloids, tannins and flavonoid which are present in the leaves extract [19]. Another study also concluded that the barks of *Acacia nilotica* possessed promising centrally and peripherally mediated locomotor depressant, skeletal muscle relaxant effects in the experimental rodent models due to flavonoids and other chemical constituents [20].

CONCLUSION

The results of the present study revealed the chrysin has significant ($P < 0.001$) and dose dependent muscle relaxant and locomotor depressant activities. Quercetin also reduced the muscle relaxant and locomotor activities but statistically not significant.

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CONFLICT OF INTEREST

The authors declare that this research does not have any conflict of interest with anyone or any institute.

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