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Research Article

Evaluation of Selected Flavonoids for the Anthelmintic and Skeletal Muscle Relaxant Activity using Animal Models

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Abstract

Most fruits and vegetables contain flavonoids, a type of phytonutrient. As well as carotenoids, they're responsible for fruits and vegetable brilliant hues. Some other phytonutrients such as flavonoids are strong antioxidants with anti-inflammatory and immune properties. There are many flavonoids, including anthocyanins, flavones, flavonols, flavonoids, and isoflavonoids. Quercetin and chrysin were chosen for the investigation. Humans and other animals can contract Helminthiasis (helminthiases), sometimes known as worm infection. Tapeworms, roundworms, and flukes are only a few of the parasites that exist. Skeletal muscle relaxants are used to treat spasticity caused by upper motor neuron syndromes and muscle discomfort or musculoskeletal spasms created by peripheral disturbances. Samples of quercetin and chrysin were generated in the presence of 0.5% SCMC suspension at concentrations of 10, 20, 30, and 40 mg/ml, and then analyzed. To keep track of photocell beam disruptions, a six-digit counter was utilized (locomotor activity). It was time to turn on the actophotometer and examine the locomotor behavior of each rat for five minutes. The basal activity levels of all the animals were recorded.

Keywords: Flavonoids, Anthelmintic, Skeletal Muscle relaxant, animal models

INTRODUCTION

Helminthiasis (plural helminthiases), also known as worm infection, is a macro parasitic disease of humans and other animals in which a part of the body becomes infected with parasitic worms known as helminths. There are many different types of parasites, which are broadly classified as tapeworms, flukes, and roundworms. They frequently live in their hosts' gastrointestinal tracts. However, they can burrow into other organs and cause physiological damage. Soil-transmitted helminthiasis and schistosomiasis are the most frequent helminthiases, both of which are considered neglected tropical illnesses[1]. Soil-transmitted helminthiases may be responsible for parasitic infections in up to a quarter of the world's population[2]. One well-known example of the soil-transmitted helminth. Helminths can be transmitted to the final host in a variety of ways. Ingestion of contaminated vegetables, drinking water, and raw or undercooked meat is the most common route of infection. Contaminated food may contain the eggs of nematodes like Ascaris, Enterobius, and Trichuris, cestodes like Taenia, Hymenolepis, and Echinococcus, and trematodes like Fasciola. Taenia (pork, beef, and venison), Trichinella (pork and bear), Diphyllobothrium (fish), Clonorchis (fish), and Paragonimus (fish) are the most common pathogens found in raw or undercooked meats (crustaceans). Hookworms (Ancylostoma and Necator) and Strongyloides are schistosomes and nematodes that can penetrate the skin directly. At last, Wuchereria, Onchocerca, and Dracunculus get transferred by mosquitoes and flies. Quercetin is a naturally occurring flavonoid that can be found in almost all edible vegetables and fruits. The Western diet contains a high amount of quercetin, approximately 15mg per day. Skeletal muscle relaxants are a broad class of drugs used to treat two sorts of underlying diseases: spasticity produced by upper motor neuron syndromes and muscular pain or spasms caused by peripheral musculoskeletal conditions[3]. Tenderness and muscular spasms are frequent symptoms of musculoskeletal diseases such as fibromyalgia, tension headaches, myofascial pain syndrome, and mechanical low back or neck discomfort. Skeletal muscle relaxants are one of several types of medications widely used to treat this disorders[4].

MATERIALS AND METHODS

Drugs and chemicals

Sigma Chemical Co. supplied quercetin and chrysin (St. Louis, MO). Lifeline Formulations Pvt Limited, Vijayawada,

India, provided albendazole as a free sample. Lifeline Formulations Pvt. Ltd. in India provided free samples of albendazole and diazepam. Sodium carboxymethyl cellulose was supplied by Finisar Chemicals Ltd. of Ahmedabad, India (SCMC). Distilled water prepared from deionized water was used throughout the investigation. Analytical chemicals and reagents were employed.

Animals

In vitro, antihelmintic activity was assessed using adult earthworms (Pheretima Posthuma). Earthworms were collected and washed with normal saline at the Vermi Compost Unit in Vijayawada, Andhra Pradesh, India [5]. In the study, earthworms measuring 6-8 cm in length were used. Sigma Chemical Co. supplied quercetin and chrysin (St. Louis, MO).

Evaluation of Anthelmintic activity

Because of its anatomical and physiological similarity to human intestinal roundworm parasites, the test was carried out in vitro on adult earthworms (Pheretima Posthuma) for preliminary evaluation of the anthelmintic activity. Quercetin and Chrysin test samples were prepared in 0.5 percent SCMC suspension at concentrations of 10, 20, 30, and 40 mg/ml. In each glass beaker containing 25 ml of the aforementioned test samples, six worms (i.e. Pheretima Posthuma) of nearly comparable size (same type) were inserted. The reference standard was albendazole (20 mg/ml), while the control was pure water. Before beginning the experiments, all of the test solutions and standard drug solutions were freshly prepared[6]. The time taken for paralysis turned incited whilst no motion of any type might be determined besides whilst the worms had been vigorously shaken. The time for the worms' death was recorded when it was determined that they did not move when shaken vigorously or submerged in warm water (50°C).

Statistical Analysis

All of the findings were put in a Table-1 and expressed as the mean + standard deviation of six worms in each. Graph Pad Prism 5 version 5.01 was used to analyze the data. A one-way ANOVA test with a confidence interval of 95 percent (ps0.05) was employed to examine the statistical significance of mean differences[7].

Evaluation of selected flavonoids and skeletal muscle relaxant activities using animal models.

Experimental animals

The animal experiments were carried out by the institutional guidelines for the care and use of laboratory animals and were approved by the animal ethics committee of KVSR Siddhartha College of Pharmaceutical Sciences (SCOPS), Vijayawada, Andhra Pradesh, India. (993/A/06/CPCSEA) The National Institute of Nutrition (NIN) in Hyderabad, Andhra Pradesh, India provided male Wistar rats weighing 180-220g[8]. Animals in the KVSR SCOPS's animal dwelling were housed in six consistent with cages and provided unrestricted access to food (Hindustan Lever, Mumbai, India) and water. For at least a week before beginning the tests, the animals were housed under typical laboratory settings (12/12 h light/darkness, 22 2°C, and 50-60% humidity).

Evaluation of skeletal muscle relaxant activity (motor coordination)

After each trial, the animals are strapped to a Rotarod (25 rpm) for 5 minutes or more, as with minor modifications. The rats had been divided into 8 corporations of six. The capsule had been given inside the following order:

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

The fall-off time from the rotating rod was measured after a half-hour of management of control, standard, quercetins, and chrysin. The difference in fall-off time from the rotating rod between the control and treated rats was used to determine muscular relaxation[9].

Evaluation of Locomotor Activity

The spontaneous locomotor activity was assessed using a photoactometer, as described by Idris et al., 2015, with slight changes. Each animal was watched for 5 minutes in a square closed field arena (30 cm x 30 cm x 30 cm) with six photocells put on the outer wall. Photocell beam disruptions were recorded using a six-digit counter (locomotor activity). The actophotometer was activated, and each rat was separately placed in the activity cage for 5 minutes to observe the locomotor activity. The baseline activity levels of all of the animals were recorded [10]. After 5 minutes of ingesting the control, standard, quercetin, and chrysin orally, the activity score was calculated. There was a difference in activity before and after the medication was given. The % decrease in motor activity was calculated.

Statistical Analysis

All statistics were computed using the Graph Pad Prism 5.0 program (San Diego, CA). The results were shown as a mean Plus standard deviation. For statistical analysis, the analysis of variance was performed, followed by Dunnett's multiple comparison tests. P values less than 0.05 have been considered significant[11].

RESULTS AND DISCUSSION

Evaluation of selected flavonoids for the anthelmintic

According to the findings, chrysin generated a dose-dependent paralytic effect much sooner, and the time to death became shorter for all of the worms shown in [Table 1]. Quercetin exhibited no paralytic effect at the dosages studied. When compared to quercetin, chrysin exhibited greater potency. Anthelmintic resistance has become a serious concern in recent years as a result of the extensive use of the broad-spectrum anthelmintic medication albendazole, and it has already been detected in livestock. To meet the World Health Organization's (WHO) global morbidity control targets for helminthiasis, novel anthelmintic drug compounds that are both effective against a broad spectrum of human nematodes and cheap are needed. The current study results reveal that chrysin has substantial anthelmintic activity when compared to albendazole. Albendazole inhibits microtubule-dependent activities such as glucose absorption by inhibiting the polymerization of helminth B-tubulin.

Table 1: Effect of quercetin and chrysin on the paralyzed and death times of earthworms

Treatment	Concentration (mg/ml)	Paralyzed Time (min)	Death time (min)
Control	-	-	-
Albendazole	20	36.3	48.5
Quercetin	10	Alive	Alive
Quercetin	20	Alive	Alive
Quercetin	30	Alive	Alive
Quercetin	40	Alive	Alive
Chrysin	10	49.6	60.1
Chrysin	20	18.4	38.7
Chrysin	30	15.5	24.2
Chrysin	40	11.2	15.3

Table 2. Effectof quercetin and chrysin on the paralyzed and death times of earthworms

Treatment	Concentration (mg/ml)	Paralyzed Time (min)	Death time (min)
Control	-	-	-
Albendazole	20	36.3	48.5
Quercetin	10	Alive	Alive
Quercetin	20	Alive	Alive
Quercetin	30	Alive	Alive
Quercetin	40	Alive	Alive
Chrysin	10	49.6	60.1
Chrysin	20	18.4	38.7
Chrysin	30	15.5	24.2
Chrysin	40	11.2	15.3

Table 3: Effect of quercetin and chrysin on the paralyzed and death times of earthworms.

Treatment	Concentration (mg/ml)	Paralyzed Time (min)	Death time (min)
Control	-	-	-
Albendazole	20	33.3	45.5
Quercetin	10	Alive	Alive
Quercetin	20	Alive	Alive
Quercetin	30	Alive	Alive
Quercetin	40	Alive	Alive
Chrysin	10	41.6	52.1
Chrysin	20	18.4	38.7
Chrysin	30	17.5	26.2
Chrysin	40	15.2	19.3

Table 4: Effect of quercetin and chrysin on the paralyzed and death times of earth worms.

Treatment	Concentration (mg/ml)	Paralyzed Time (min)	Death time (min)
Control	-	-	-
Albendazole	20	31.96±5.13	44.6±5.13
Quercetin	10	Alive	Alive
Quercetin	20	Alive	Alive
Quercetin	30	Alive	Alive
Quercetin	40	Alive	Alive
Chrysin	10	44.26±4.61	54.76±4.61
Chrysin	20	16.46±3.52	36.7±3.46
Chrysin	30	14.83±3.05	23.53±3.05
Chrysin	40	11.86±3.05	15.96±3.05

Evaluation of selected flavonoids and skeletal muscle relaxant activities using animal models.

Rotarod Test

When compared to the control, chrysin significantly reduced the amount of time the animals spent on the rotating rod (P0.000). The findings are summarised in [Table 1]. The usual medication (diazepam) showed a significantly significant impact when compared to the control (P0.000). When compared to the control, three distinct dosages of chrysin (20, 40, and 60 mg/kg) exhibited a dose-dependent increase in muscular relaxation, specifically 193.83 + 6.62 and 31.00 7.72 after 90 minutes of treatment. At a dosage of 60 mg/kg, chrysin produced the greatest amount of muscular relaxation. The Rotarod test indicated that chrysin decreased the motor coordination of the tested animals considerably. The amount of time spent on the rotating rod was similarly reduced by quercetin, albeit the difference was not statistically significant.

Actophotometer

Locomotor activity research found that chrysin substantially (0 > d) decreased locomotor activity in a dosage and time-dependent manner. The activity intensified as the clock reached 90 minutes. The results are summarised in [Table 2]. After 90 minutes, the percentage of locomotor activity reduction with diazepam (10 mg/kg, p. o.) was 89.87, showing a highly significant (P0.000) decrease in locomotor activity as compared to the control. At a dosage of 60 mg/kg, chrysin produced the greatest amount of muscular relaxation. None of the three quercetin dosages (20, 40, or 60 mg/kg, p. o.) resulted in a statistically significant reduction in locomotor activity.

Plant flavonoids have prompted a rise in popular and scientific interest in recent years due to their purported health advantages. Flavonoids are specialized metabolites found in plants that comprise big groups of low-molecular-weight polyphenolic chemicals with biological characteristics beneficial to human health. So far, about 5000 distinct flavonoids have been found. According to nutritionists, the typical daily consumption of flavonoids by people on a regular diet is 1-2 g. Flavonoids are naturally occurring polyphenols with hydroxylation and substitution patterns that give birth to several subclasses including flavanones, anthocyanidins, flavonols, flavones, catechins (or flavanols), isoflavones, dihydroflavonols, and chalcones.

Several in vitro and in vivo investigations have indicated that chrysin possesses anti-disease properties. Chrysin has a wide range of biological and pharmacological activities, including antioxidant, anti-inflammatory, anticancer, neuroprotective, colon protective, nephroprotective, antidiabetic, hypolipidemic, antiarthritic, antiasthmatic, antidepressant, hepatoprotective, cardioprotective, and antiviral properties. Flavonoids show potential as skeletal muscle relaxants. The current investigation discovered that chrysin exhibited muscle relaxant and locomotor depressing effects in experimental animals. Previous studies indicated that the methanolic extract of Basella Alba exhibits antidepressant-like effects as well as skeletal muscle relaxant activity. The action might be attributable to the alkaloids, tannins, and flavonoids present in the leaf extract. Another study indicated that flavonoids and other chemical components in the bark of Acacia nilotica displayed potential centrally and peripherally mediated locomotor depression, skeletal muscle relaxant effects in experimental mouse models.

Table 5.Effectof quercetin and chrysin on muscle relaxant activity in rats

Treatment	Fall off time(seconds) before treatment						
	R1	R2	R3	R4	R5	R6	Mean ±SD
Control	196	189	178	190	206	188	191.17±9.30
Diazepam (10mg/kg)	178	161	168	175	159	173	169.00±7.72
Quercetin (20mg/kg)	186	167	176	171	184	169	175.50±7.97
Quercetin (40mg/kg)	193	172	183	191	176	178	182.17±8.42
Quercetin(60mg/kg)	173	154	156	163	170	168	164.00±7.72
Chrysin(20mh/kg)	160	177	163	170	180	165	169.17±7.99
Chrysin(40mg/kg)	170	185	171	180	187	175	178.00±7.16
Chrysin60mg/kg)	185	188	202	192	199	197	193.83±6.62

Table 6.Effects of quercetin and chrysin on muscle relaxant activity in rats after 30 mins of diazepam treatment.

Treatment	Fall off time(seconds) before treatment						
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ±SD**
Control	175	174	177	182	191	187	181.00±6.90
Diazepam (10mg/kg)	96	94	81	86	77	76	85.00±8.53
Quercetin (20mg/kg)	161	175	178	159	168	173	169.00±7.72
Quercetin (40mg/kg)	181	179	166	171	162	161	170.00±8.53
Quercetin (60mg/kg)	164	159	154	161	144	145	154.50±8.41
Chrysin (20mh/kg)	100	110	119	103	120	101	108.83±8.98
Chrysin (40mg/kg)	86	106	96	92	89	101	95.00±7.54
Chrysin 60mg/kg)	72	92	73	82	78	91	81.33±8.66

Table 7: Effects of quercetin and chrysin on muscle relaxant activity in rats after 60 mins of diazepam treatment.

Treatment	Fall off time(seconds) before treatment						
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ±SD**
Control	184	181	169	174	167	164	173.17±7.99
Diazepam (10mg/kg)	34	39	47	29	49	32	38.33±8.19
Quercetin (20mg/kg)	164	145	154	162	144	159	154.67±8.57
Quercetin (40mg/kg)	180	173	187	190	175	185	181.67±6.80
Quercetin (60mg/kg)	158	139	143	141	148	156	147.50±7.97
Chrysin (20mh/kg)	52	67	69	73	53	62	62.67±8.64
Chrysin (40mg/kg)	45	63	54	47	64	46	53.17±8.61
Chrysin 60mg/kg)	40	57	52	47	59	38	48.83±8.70

Table 8.Effects of quercetin and chrysin on muscle relaxant activity in rats after 90 mins of diazepam treatment

Treatment	Fall off time(seconds) before treatment						
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ±SD**
Control	181	164	180	171	176	162	172.33±8.07
Diazepam (10mg/kg)	34	25	18	5	17	35	24.00±8.81
Quercetin (20mg/kg)	148	150	131	136	141	132	139.67±8.07
Quercetin (40mg/kg)	156	170	165	157	175	158	163.50±7.82
Quercetin (60mg/kg)	143	151	160	144	161	146	150.83±7.99
Chrysin (20mh/kg)	40	36	45	52	35	55	43.83±8.33
Chrysin (40mg/kg)	42	37	25	32	23	37	32.67±7.45
Chrysin 60mg/kg)	30	23	35	21	37	40	31.00±7.72

Table 9: 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 10mg/kg)	169.00±7.72	85.00±8.53	38.33±8.19	24.00±8.81	49.704	77.319	85.798
Quercetin(20mg/kg)	175.50±7.97	169.00±7.72	154.67±8.57	139.67±8.07	3.703	11.868	20.415
Quercetin(40mg/kg)	182.17±8.42	170.00±8.53	181.67±6.80	163.50±7.82	6.680	0.274	10.248
Quercetin 60mg/kg)	164.00±7.72	154.50±8.41	147.50±7.97	150.83±7.99	5.792	10.060	8.030
Chrysin (20mg/kg)	169.17±7.99	108.83±8.98	62.67±8.64	43.83±8.33	35.668	62.954	74.091
Chrysin (40mg/kg)	178.00±7.16	95.00±7.54	53.17±8.61	32.67±7.45	46.629	70.129	81.646
Chrysin (60mg/kg)	193.83±6.62	81.33±8.66	48.83±8.70	31.00±7.72	58.040	74.80	84.006

% reduction in fall off time= $(W_a-W_b)/W_a\times100\%$.

Where W_a and $W_b$are the mean fall-off times before and after treatment, respectively.

RESULTS WITH ACTOPHOTOMETER

Table 10: Effects of quercetin and chrysin on locomotor activity in rats

Treatment	Actophotometer score before treatment						
	R1	R2	R3	R4	R5	R6	Mean ±SD
Control	215	205	208	225	222	204	213.17±8.93
Diazepam	168	171	178	185	188	177	177.83±7.73
Quercetin (20mg/kg)	185	180	177	190	195	175	183.67±7.79
Quercetin (40mg/kg)	170	165	166	180	167	160	168.00±6.72
Quercetin(60mg/kg)	186	487	196	184	206	191	191.67±8.21
Chrysin(20mh/kg)	147	154	149	144	148	164	151.00±7.16
Chrysin(40mg/kg)	130	125	120	128	140	127	128.33±6.65
Chrysin60mg/kg)	137	145	138	155	147	135	142.83±7.60

Table 11.Effects of quercetin and chrysin on locomotor activity in rats after 30 mins of diazepam treatment.

Treatment	Actophotometer score before treatment						
	R1	R2	R3	R4	R5	R6	Mean ±SD
Control	182	192	184	202	185	187	188.67±7.37
Diazepam	66	49	56	47	51	46	52.50±7.50
Quercetin (20mg/kg)	168	171	176	178	173	188	175.67±7.00
Quercetin (40mg/kg)	163	147	143	146	153	144	149.33±7.55
Quercetin(60mg/kg)	174	175	194	177	179	184	180.50±7.50
Chrysin(20mh/kg)	100	83	80	85	90	87	87.50±7.01
Chrysin(40mg/kg)	76	73	84	83	75	93	80.67±7.50
Chrysin60mg/kg)	55	60	65	57	58	75	61.67±7.37

Table 12.Effects of quercetin and chrysin on locomotor activity in rats after 60 mins of diazepam treatment.

Treatment	Actophotometer score after 60 mins of treatment						
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ±SD**
Control	174	169	167	164	165	184	170.50±7.50
Diazepam	19	29	20	23	39	25	25.83±7.39
Quercetin (20mg/kg)	174	155	164	167	157	154	161.83±7.88
Quercetin (40mg/kg)	140	143	152	150	145	160	148.33±7.23
Quercetin (60mg/kg)	168	171	188	175	178	172	175.33±7.09
Chrysin (20mh/kg)	26	43	29	25	23	33	29.83±7.33
Chrysin (40mg/kg)	35	52	38	32	42	37	39.33±7.03
Chrysin (60mg/kg)	21	25	24	31	23	41	27.50±7.42

Table 13.Effects of quercetin and chrysin on locomotor activity in rats after 90 mins of diazepam treatment.

Treatment	Actophotometer score after 90 mins treatment						
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**Mean ±SD**
Control	166	161	176	158	156	159	162.67±7.37
Diazepam	11	21	14	15	31	16	18.00±7.16
Quercetin (20mg/kg)	147	149	157	151	137	150	148.50±6.57
Quercetin (40mg/kg)	135	138	139	145	147	155	143.17±7.33
Quercetin (60mg/kg)	181	164	191	165	171	166	173.00±10.83
Chrysin (20mh/kg)	11	14	31	15	17	21	18.17±7.11
Chrysin (40mg/kg)	35	20	15	18	25	17	21.67±7.37
Chrysin 60mg/kg)	28	11	15	18	20	8	16.67±7.09

Table 14.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 (10mg/kg)	177.83±7.73	52.50±7.50	25.83±7.39	18.00±7.16	70.47	85.47	89.87
Quercetin (20mg/kg)	183.67±7.79	175.67±7.00	161.83±7.88	148.50±6.57	4.30	11.88	19.14
Quercetin (40mg/kg)	168.00±6.72	149.33±7.55	148.33±7.23	143.17±7.33	11.11	11.70	14.82
Quercetin (60mg/kg)	191.67±8.21	180.50±7.50	175.33±7.09	173.00±10.83	5.82	8.50	9.70
Chrysin (20mg/kg)	151.00±7.16	87.50±7.01	29.83±7.33	18.17±7.11	42.05	80.24	88.01
Chrysin (40mg/kg)	128.33±6.65	80.67±7.50	39.33±7.03	21.67±7.37	37.14	69.35	83.12
Chrysin (60mg/kg)	142.83±7.60	61.67±7.37	27.50±7.42	16.67±7.09	56.82	80.7	88.33

CONCLUSION

The findings of this investigation revealed that chrysin had substantial anthelmintic activity when compared to quercetin and albendazole. As a result, given the development of a novel medication for the treatment of helminthiasis, more study should be extremely beneficial in determining the efficacy of employing chrysin as a new putative anthelmintic therapy.

The findings of this investigation revealed that chrysin has considerable (P 0.001) and dose-dependent muscle relaxant and motor depression action. Quercetin also reduced muscle relaxant and motor activity, but this was not statistically significant.

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Conflict of interest

The authors attest that they have no conflict of interest in this study.

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