

Evaluation of Quercetin and Chrysin for Anthelmintic Activity Using Adult Indian Earthworm Model

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ABSTRACT

Objective: Helminths infections are also among the most common infections in human, affecting a large proportion of the world's population in developing countries and produce a global burden of disease. *Pheretima posthuma* a helminth is commonly known as earth-worms. Quercetin and chrysin are the flavonoids, which were evaluated for the anthelmintic activity using *Pheretima posthuma* model (Indian earthworm). Albendazole was used as the standard reference. **Methods:** Four concentrations (10, 20, 30 and 40 mg/ml) of these flavonoids were used for this study which involved the determination of time of paralysis (vermifuge) and time of death (vermicidal activity) of the worms. **Results:** Chrysin not only paralyzed but also killed the earthworms dose-dependently but Quercetin not showed any paralytic effect (i. e. animals are alive). Chrysin showed maximum vermicidal activity at the concentration of 40 mg/ml followed by 30 mg/ml. Observations were comparable with the standard drug at concentration of 20 mg/ml. **Conclusion:** On the basis of the observations it is concluded that Chrysin has a significant anthelmintic activity when compared to the Quercetin.

Keywords: Chrysin, Quercetin, Anthelmintic activity, *Pheretima posthuma*, Albendazole.

INTRODUCTION

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia¹. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas². The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases³. Hence there is an increasing demand towards natural anthelmintics.

Flavonoids are a group of polyphenolic compounds which are widely distributed throughout the plant kingdom and about 3000 varieties of flavonoids are known⁴. Many have low toxicity in mammals and some of them are widely used in medicine⁵. Flavonoids exhibit several biological effects such as anti-hepatotoxic, anti-inflammatory, antiulcer activity⁶, antiatherosclerotic, antitumor, antithrombogenic, antiosteoporotic, antiviral⁷, antibacterial⁸ and antifungal activities⁹. Quercetin is flavonol from the flavonoid group of polyphenols. Chrysin, also called 5, 7-dihydroxyflavone, is a flavones. Previous studies indicated that the aqueous and ethanolic extracts of *Barleria buxifolia* have shown a good anthelmintic activity due to presence of alkaloids,

flavonoids, tannins and steroids which have been associated with anthelmintic activity¹⁰. Based on this, an attempt has been made to evaluate the anthelmintic potential of flavonoids (quercetin and chrysin) in the present study.

MATERIALS AND METHODS

Drugs and chemicals

Quercetin and chrysin were purchased from Sigma Chemical Co. (St. Louis, MO). Albendazole was obtained as gift sample from Lifeline Formulations Pvt. Limited, Vijayawada, India. 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.

Animals

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity in vitro. Earthworms were collected from Vermi Compost Unit located at Vijayawada, Andhra Pradesh, India and washed with normal saline. The earthworms of 6-8 cm in length were used in the study.

Evaluation of Anthelmintic activity

The anthelmintic assay was carried out as per the method of Dash et al. 2002¹¹. The assay was performed in vitro using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the

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Table 1: Effect of quercetin and chrysin on the paralyzed and death times of earthworms (N=6).

Treatment	Concentration (mg/mL)	Paralyzed Time (Minutes)	Death Time (Minutes)
Control	-	-	-
Albendazole	20	31.3 ± 2.53	43.5 ± 4.68
Quercetin	10	Alive	Alive
Quercetin	20	Alive	Alive
Quercetin	30	Alive	Alive
Quercetin	40	Alive	Alive
Chrysin	10	45.6 ± 3.48	56.1 ± 4.32
Chrysin	20	15.4 ± 1.62**	35.7 ± 3.14**
Chrysin	30	13.5 ± 0.85**	22.2 ± 2.62**
Chrysin	40	10.2 ± 0.63**	14.3 ± 1.48**

intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity¹². Quercetin and Chrysin test samples were prepared at the concentrations, 10, 20, 30 and 40 mg/ml in 0.5% SCMC suspension. Six worms (i.e. *Pheretima posthuma*) of approximately equal size (same type) were placed in each glass beaker containing 25 ml of above test samples. Albendazole (20 mg/ml) was used as reference standard and distilled water as control. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C).

Statistical Analysis

All the results were shown in Table.1 and expressed as a mean ± SD of six worms in each group. Analysis of the data was performed using Graph Pad Prism 5 version 5.01. One way ANOVA test with confidence interval at 95% (* = $p \leq 0.05$) was used to determine the statistical significance of the differences of the mean.

RESULTS AND DISCUSSION

From the observations, chrysin has produced dose dependent paralytic effect much earlier and the time to death was shorter for all worms shown in Table 1. But quercetin has no effect on earth worms (i. e. Alive). Quercetin has not showed any paralytic effect at the selected doses. Chrysin exhibited more potent activity when compared to quercetin. Due to the heavy use of broad spectrum anthelmintic drug albendazole anthelmintic resistance is a major challenge today, which is already being noticed in live stocks. Therefore, development of new anthelmintic drug molecules that are effective against broad spectrum for human nematode as well as inexpensive to achieve World Health Organization (WHO) goals for global control of morbidity caused by helminthiasis is mandatory¹³. The present study results revealed that chrysin has significant anthelmintic activity when compared to albendazole. Albendazole acts by inhibiting the polymerization of helminth β -tubulin, and thus interfering with microtubule dependent functions like glucose uptake¹⁴.

Previously, Rohini et al. (2011) conducted a study on earthworms with *Zingiber officinale* and *Curcuma longa* and identified that the anthelmintic activity of these plants may be due to the synergetic effect of active phyto-constituents i.e. alkaloids, saponins, flavonoids, terpenes, steroids, etc¹⁵. In another study, Purna et al. (2014) showed that the aqueous and ethanolic extracts of *Barleria buxifolia* have shown a good anthelmintic activity due to presence of alkaloids, flavonoids, tannins and steroids which have been associated with anthelmintic activity¹⁰.

CONCLUSION

The present study results revealed that chrysin has significant anthelmintic activity when compared to quercetin and albendazole. Therefore, in view of the new drug development for the treatment of helmintheasis further studies shall prove highly beneficial to establish the effectiveness of the use of chrysin as a novel putative anthelmintic drug.

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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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