The Therapeutic Effect of Flavone on the Muscular Phase of *Trichinella spiralis* Infection in a Murine Model

Original Article

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ABSTRACT

Introduction: *Trichinella spiralis* is the cause of trichinosis disease. The recommended treatments, Mebendazole or Albendazole, have many side effects that can hardly eliminate encysted larvae. Flavone, a plant-derived compound, has shown promising anti-parasitic, antioxidant, and immunomodulatory properties, offering a natural and safer alternative.

Objective: The current study aims to evaluate the potential therapeutic effect of Flavone alone and in combination with Mebendazole on the muscular phase of *Trichinella spiralis* infection in experimentally infected mice.

Methodology: This experiment was performed on 20 mice (Swiss.albino) divided into Group-I, a healthy control; Group-II, infected-untreated; Group-III, infected and Mebendazole-treated; Group-IV, infected and Flavone-treated; and Group-V, infected and Flavone+ Mebendazole-treated. Drugs assessment was done on day 35 post-infection (PI) and involved counting of the encysted larvae and the histopathological evaluation of the muscle specimens.

Results: Mebendazole + Flavone-treated mice recorded significant reductions in the mean counts of the encysted larva with percentage of reduction (efficacy) 94.53% followed by the Flavone-treated mice (83.95%). Yet, the efficacy of Mebendazole in treated mice was 74.13%, P value < 0.05. The nurse cell length and inflammatory cell counts recorded the least values in the Mebendazole + Flavone-treated mice followed by the Flavone-treated group compared to their values in Group-II and Group-III.

Conclusions: Flavone seemed to be an effective and safe alternative to Mebendazole. Nevertheless, mebendazole + Flavone revealed more promising results against *T. spiralis* infections than sole therapies.

Key Words: Flavone, mebendazole, muscular phase, *trichinella spiralis*.

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INTRODUCTION

Human trichinellosis is one of the most prevalent parasitic zoonoses globally, arising from nematode infections within the *Trichinella* genus. The main way the infection spreads is by consuming undercooked meat that contains larvae[1].

This parasite has the potential to cause significant health complications. People who are infected often suffer symptoms such as muscle pain, fever and gastrointestinal disturbances. If left untreated, trichinellosis can result in serious complications. Infection remains of a significant concern, especially in regions where undercooked or raw meat consumption is common[2].

The current treatment of *Trichinella* infection typically involves using antiparasitic chemical Medications, especially benzimidazole derivatives like Mebendazole or Albendazole to eliminate the parasite[2].

The current treatment of *Trichinella* infection typically involves using antiparasitic chemical medications, especially benzimidazole derivatives like Mebendazole or Albendazole to eliminate the parasite[2]. The anti-helminthic spectrum of Mebendazole and Albendazole are similar[3]. These medications and their metabolites directed against parasites in the intestinal and extra-intestinal tissues[4]. However, these medications are associated with various side effects and are inefficient in encysted larvae eradication. Furthermore, some show minimal absorption and high resistance. These restrictions highlight the necessity for new, efficient, and secure treatments for trichinellosis[3,4].

Medicinal herbs offer a hopeful alternative or adjuvant, as they are more affordable, less harmful, and lack the adverse side effects of chemical drugs. One particular compound found in plants, known as Flavone, a subclass of the Flavonoids family, is recognized for various activities, for example, possessing antioxidant, anti-

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inflammatory, antimicrobial, antiparasitic, and anticancer properties[5,6,7,8,9]. To the best of our knowledge, the effect of Flavone on trichinellosis has not been assessed yet.

As a step towards this family of Phytotherapy, this study aimed to assess the effects of Flavone solely and in combination with mebendazole treatment on *T. spiralis* infection in the muscular phase in murine models through evaluating parasitological and histopathological parameters.

METHODOLOGY

Trial animals

The experiment was performed on 20 mice (Swiss. albino), 30 ± 5 grams weight and 9–11 weeks of age. The animals were kept in the biological unit of TBRI in fitted, aired cages at 24°C temperature. They were held onto a routine diet including twenty four percent protein, four percent fat, and about five percent fiber and water. Safe disposal for the mice's waste and for the dead mice was done.

Parasites isolation and preparation of the inoculums

larvae of *T. spiralis* were taken from infested mice, provided by the Medical Parasitology Department, TBRI. Stool examination was done to exclude any other intestinal parasitic infection. murine muscles were cut into small pieces and placed in a glass beaker containing artificial gastric juice. The composition of this juice is 1% pepsin (w/v) mixed with one percent HCl (v/v) in distilled water. The mixture was incubated overnight at 37°C to ensure complete muscle digestion, facilitating the larva extraction[10]. A copper sieve with 50 mesh per inch was initially used to filter larger particles, followed by a two hundred mesh/inch finer sieve to capture all the larvae that excysted. Then the larvae were rinsed two to three times with distilled water and placed in a flask for approximately 30 minutes to allow sedimentation. Afterward, the supernatant was gently removed with a 10 ml syringe, and the live larvae (coiled and mobile) in the sediment were counted using a hemocytometer. The inoculums for each mouse (from group II to group V) were adjusted to contain about 500 larvae[10].

Study design

The mice were split into five groups of 4 mice each:

- **Group I:** Non-infected non-treated (healthy control).
- **Group II:** Infected non-treated.
- **Group III:** Infected treated with Mebendazole.
- **Group IV:** Infected treated with Flavone.
- **Group V:** Infected treated with Mebendazole and Flavone.

Drug administration

Mebendazole was given in a dosage of fifty milligram per kilogram after the infection on day 30 for five consecutive days[11,12], and Flavone was administered in a dosage fifty milligram per kilogram after the infection on day 30 for five consecutive days[13].

Testing the drug effects

Parasitological examination. The recognition and enumeration of encapsulated larvae were performed after 35 days from the infection, where the diaphragms and thighs of the diseased mice were chopped into tiny pieces, weighed, and compressed on microscopic slides for larval counting under a light microscope (low magnification power x10). For each treatment, the therapeutic effects were assessed by the mean counts of encysted larvae per gram muscle tissue[11]. The effectiveness for each therapy was computed according to an equation previously described by García *et al*.[14].

Percentage of reduction (efficacy) =

$$\frac{\text{(mean counts of encysted larvae in GroupII-mean counts of encysted larvae in the treated group)}}{\text{(mean counts of encysted larvae in GroupII)}} \times 100$$

Histopathological assessment. Muscle samples were gathered from mice sacrificed on day 35 after the infection. The samples were placed in 10% buffered formalin that is neutral for one day, then de-hydrated in increasing concentrations of Ethanol, cleaned with xylene, implanted in blocks of paraffin, and sectioned into 5 µm thick slices via microtome. followed by staining with Haematoxylin and Eosin (H&E) for histopathological examination to evaluate the extent of the disease and treatment effects[10,15], where sections were analyzed using an Olympus CX41RF microscope, digital images were captured via an Olympus SC30 camera, and muscle sections were evaluated for nurse cell length, and inflammatory cell counts per high-power field (40× magnification), with the assessment conducted in 10 power fields per mouse and mean values calculated.

Statistical analysis of data

Pre-coded data was processed and statistically analysed by utilizing the statistical package of the social science software (SPSS), version 21. Data has been summarized using SD and mean for quantitative variables that are distributed normally or median and IQR for abnormal distributed quantitative variables. Number and percentage were utilized for description of qualitative variables. The test of Chi-square was utilized to compare variables that are qualitative, the non-dependent T-test has been utilized for regularly distributed quantitative variables among groups, and Nonparametric Mann-Whitney tests were applied for non-normally distributed quantitative data. The paired sample t-test and Wilcoxon Signed-Rank test have

been utilized for the examination of paired data. Additional statistical tests were utilized if required. P-values less than 0.05 were deemed statistically significant[16,17,18].

Ethical considerations

The protocol of this study was agreed by the ethical committees of The Armed Forces College of Medicine (AFCM) serial number (343) and Theodor Bilharz Research Institute (TBRI) serial number PT (832). All trials were carried out agreeing to the guidelines of Clinical and Laboratory Standards Institute (CLSI).

RESULTS

Parasitological results

The drugs' impact through the phase of muscular invasion was assessed in each group by enumerating the larvae/gram of muscle tissue/group, (Figure 1). The mean larvae count / group and percentage of reduction are presented in (Table 1).

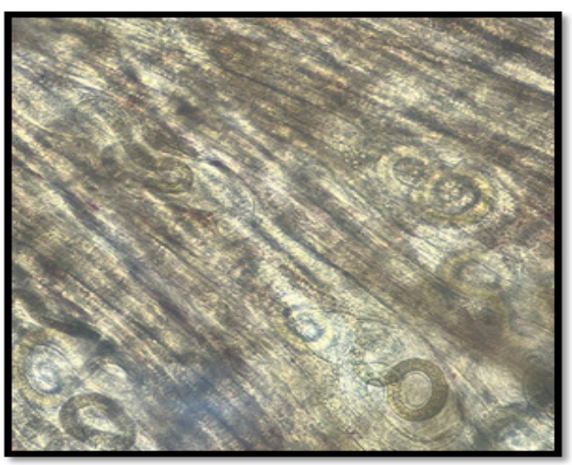


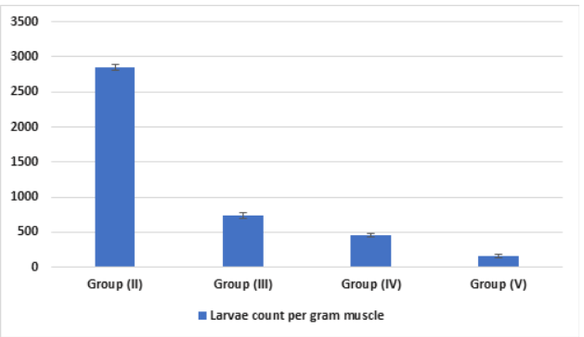
Fig. 1: Larvae of *T. spiralis* under light microscope (10× magnification)

Table 1: *Trichinella* larvae count expressed as Mean ± SD/gram muscle and percentage of reduction in infected non-treated and infected treated groups

Groups	Larvae count (Mean ± SD)	Percentage of reduction
Group (II) (Infected non-treated)	2846.5 ± 37.76	-
Group (III) (Infected treated with Mebendazole)	736.3 ± 48.46 #	74.13%
Group (IV) (Infected treated with Flavone)	457 ± 24.52 #$	83.95%
Group (V) (Infected and treated with Mebendazole and Flavone)	155.8 ± 30.30 #$@	94.53%

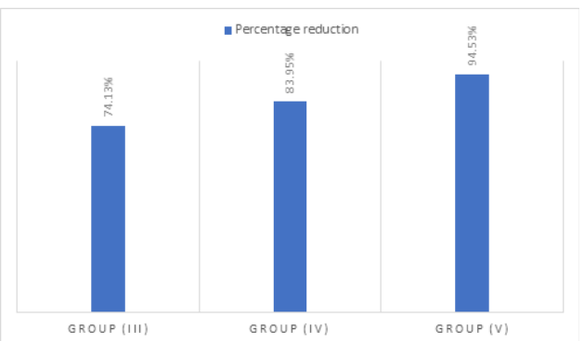
#: There is a difference which is significant when compared to (Group II) (*P-value* < 0.05), $: There is a difference which is significant when compared to (Group III) (*P-value* < 0.05), @: There is a difference which is significant when compared to (Group IV) (*P-value* < 0.05)

The mean larvae count / muscle in infected treated groups (III, IV, and V) was reduced to (736.3±48.46, 457±24.52, and 155.8±30.30, respectively) with a statistically significant difference (*P value* = < 0.05) versus the infected nontreated (2846.5 ± 37.76) (Graph 1).



Graph 1: Mean number of Trichinella larvae / gram muscle tissue / group among the studied groups

As demonstrated in (Table 1), the highest percentage of reduction in larvae count (94.53%) was found in the group infected treated with Mebendazole + Flavone, followed by the group infected and treated with Flavone solely (83.95%), with statistically significant difference (*P value* < 0.05) versus that group infected treated with Mebendazole (74.13%) (Graph 2).



Graph 2: Percentage reduction of Trichinella spiralis larvae count among infected treated groups

Histopathological results

Haematoxylin and eosin stained-muscle sections were assessed for nurse cell length and inflammatory cell count/ muscle field, (Figure 2).

Concerning the nurse cell length, the infected non-treated group measured (374.62µm ± 6.44). It was decreased to (247.68µm ± 9.26) in infected treated with Mebendazole sole therapy. The group infected treated with Flavone solely showed further decreases in the nurse cell length (191.36 µm± 1.25). The shortest length was scored with that group infected treated with both Mebendazole + Flavone (110.96µm ± 8.96), with a statistically significant difference (*P value* < 0.05) (Table 2, Graph 3).

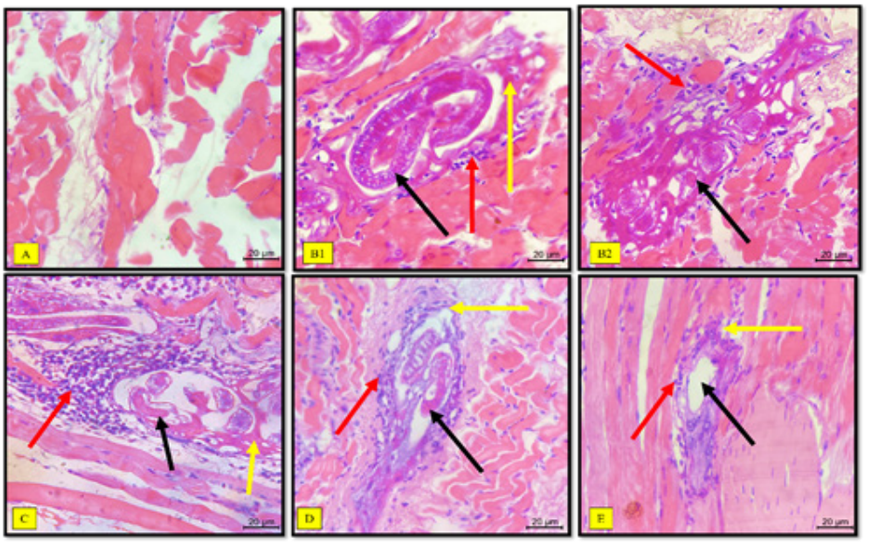
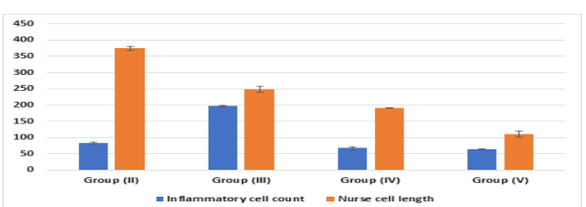


Fig. 2: H & E-stained muscular sections from the five groups showing differences in the nurse cell length, and inflammatory cell count. A. Group (I): transverse sections of normal muscle appearance with regular muscle bundles. B1. group (II): showing living Trichinella larvae (black pointer) within the nurse cell (yellow pointer) surrounded by infiltrates of lymphoplasmacytic cells (red pointer). B2. group (II) showing cluster of larvae (black pointer) bounded by moderate inflammatory cellular infiltrates (red pointer). C. group (III): showing Trichinella larva (black pointer) within the nurse cell (yellow pointer) surrounded by intense infiltrates of lymphoplasmacytic cells (red pointer). D. group (IV): showing degenerated Trichinella larvae (black pointer), disrupted nurse cell (yellow pointer) surrounded by mild lymphoplasmacytic cellular infiltrate (red pointer). E. Group (V): showed normal muscle bundles, disrupted nurse cell (yellow pointer), no larvae (black pointer), and mild inflammatory cells (red pointer) (40× magnification).

Table 2: Infected non-treated group compared to the infected treated groups (III, IV, V) regarding nurse cell length and inflammatory cell count expressed as Mean ± SD/muscle field (40× magnification)

Groups	Nurse cell length (µm) (Mean ± SD)	Inflammatory cell count (Mean ± SD)
Group (II) (Infected non-treated)	374.62 ± 6.44	83.5 ± 3.03
Group (III) (Infected treated with Mebendazole)	247.68 ± 9.26 #	196.7 ± 2.21 #
Group (IV) (Infected treated with Flavone)	191.36 ± 1.25 #$	67.6 ± 4.6 #$
Group (V) (Infected and treated with Mebendazole and Flavone)	110.96 ± 8.96 #$@	64.3 ± 1.89 #$

#: There is a difference which is significant when compared to (Group II) (*P-value* < 0.05), $: There is a difference which is significant when compared to (Group III) (*P-value* < 0.05), @: There is a difference which is significant when compared to (Group IV) (*P-value* < 0.05)



Graph 3: Inflammatory cell counts and nurse cell length of *T. spiralis* larvae/muscle field between the infected non-treated and infected treated groups (40× magnification)

The inflammatory cell count recorded the highest count in the infected mice treated with Mebendazole sole treatment (196.7 ± 2.21), followed by the infected non-treated group (83.5 ± 3.03), while in the infected mice treated with Flavone sole therapy it was (67.6 ± 4.6), and in infected- (Mebendazole + Flavone) treated animals was (64.3 ± 1.89) with a statistically significant difference (*P value* < 0.05) (Table 2, Graph 3).

The group infected and treated with Mebendazole + Flavone and the group infected and treated with Flavone sole therapy showed a statistically significant reduction compared with the infected Mebendazole-treated animals and the infected non-treated group, (Table 2).

DISCUSSION

In this study, concerning parasitological assessment during muscular phase, it was found that the combination of Mebendazole + Flavone caused significant larval reduction achieving the best results in treating muscular trichinosis with a (94%) reduction in larvae count. This was followed by the Flavone-treated subgroup with an (83%) reduction. Lastly, Mebendazole-treated subgroup recorded the lowest percentage of reduction (74%).

In accordance with this study, Maged *et al*.[19] reported the profound impact of the combination therapy (kaempferol (a flavonoid) + Albendazole) on the parasite burden in muscle tissues with percentage of reduction (85%) followed by kaempferol (78%) then the group treated with Albendazole (75%).

Huang *et al*.[20] showed that Sanguinarine (flavonoid derivative) could significantly reduce the burden of worms through all stages of the disease in mice. Similarly, El-Melegy *et al*.[21] reported that Mebendazole as sole therapy resulted in a (40.18%) percentage of reduction. Many studies likewise, documented the lower percentage of reduction when using Albendazole[10,22]. These low percentages of reduction could be attributed to lower doses used and the duration of treatment[23,24]. On the other hand, Chai *et al*.[25] stated that Mebendazole demonstrated superior efficacy in reducing muscle larvae in mice, achieving a (96%) reduction rate.

Regarding histopathological assessment, in this work, the infected non-treated subgroup revealed numerous larvae encysted in thick capsules, surrounded by inflammatory cellular infiltrates. It was also observed that the Mebendazole-treated subgroup induced a marked inflammatory response, leading to a reduction in nurse cell length and disruption of the larvae.

Similarly, El-Melegy *et al*.[21] and Fredericks *et al*.[26] found that Mebendazole caused partial fragmentation in the encapsulated larvae structure with heavy lymphocytic infiltration. Likewise, Attia *et al*.[27] and El-Wakil *et al*.[28,29] reported a marked inflammatory reaction around the thick capsules and between muscle fibers in *T. spiralis* infected-non-treated group and infiltration of inflammatory cells surrounding and invading the larval capsules in Albendazole-treated group.

On the other hand, in this study Flavone elicited a minimal inflammatory response. However, it significantly reduced the length of the nurse cell. This disrupting effect emphasizes the pharmacological activity of the Flavonoids to target intracellular pathogens[30].

The combined treatment intensively disrupted the inflammatory capsule surrounding the infected myocyte with the lowest counts of immune cells. Also, the larvae appeared as thinned remnants.

Similar histopathological alterations were reported by Attia *et al*.[27] when Albendazole was applied in combination with myrrh, which contains flavonoids. Also, El-Wakil *et al*.[29] used Albendazole in conjugation with berberine, a plant-derived compound, and showed a mild inflammatory reaction with increased disruption of nurse cell structures.

CONCLUSIONS

The current study showed that the combined treatment (Mebendazole + Flavone) was the most effective in *T. spiralis* infection muscular phase. The combined treatment reduced larvae burden, shortened nurse cell length, and minimized inflammatory cells. Flavone alone reduced larvae burden with mild inflammatory cell infiltration. Mebendazole alone showed the highest inflammatory cell infiltration, still had the lowest impact on *T. spiralis* nurse cell.

Prospects for further evaluation of Flavone on the intestinal phase is recommended.

ABBREVIATIONS

T. spiralis: *Trichinella spiralis,* PI: Post infection, **C:** Celsius, **H&E:** Haematoxylin and Eosin , **AFCM:** Armed Forces college of Medicine, **TBRI:** Theodor Bilharz Research Institute .

AUTHORS' CONTRIBUTIONS

RK conceptualized the study. RK and EA designed the study. RK and EA designed the data collection tool. MA and ES carried out data collection. MA and ES performed the data analysis and interpretation. MA wrote the original draft. RN, EA and SS revised the article before submission.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Abuelenain GL, Fahmy ZH, Elshennawy AM, Selim EH, Elhakeem M, Hassanein KM, Awad SM. Phenotypic changes of Trichinella spiralis treated by Commiphora molmol, Lepidium sativum, and Albendazole: in *vitro* study. Helminthologia. 2022 May 4;59(1):37.

2. Pozio E, Murrell KD. Systematics and epidemiology of Trichinella. Advances in parasitology. 2006 Jan 1;63:367-439.

3. Xiao SH, Xue J, Xu LL, Zhang YN, Qiang HQ. Comparative effect of mebendazole, albendazole, tribendimidine, and praziquantel in treatment of rats infected with Clonorchis sinensis. Parasitology research. 2011 Mar;108:723-30.

4. Mohammed OM, Saif Al Deen MK, Noori SS, Salam I. Efficacy and Safety of Mebendazole in the Treatment of Intestinal Helminth Infections. South Asian Res J Pharm Sci. 2024;6(5):162-8.

5. Semwal, R.B., Semwal, D.K., Combrinck, S., Trill, J., Gibbons, S. and Viljoen, A., 2019. Acacetin—A simple flavone exhibiting diverse pharmacological activities. Phytochemistry Letters, 32, pp.56-65.

6. Ballard CR, Junior MR. Health benefits of flavonoids. InBioactive compounds 2019 Jan 1 (pp. 185-201). Woodhead Publishing.

7. Ghosh D, Mukherjee PK, editors. Natural medicines: clinical efficacy, safety and quality. CRC Press; 2019 Jul 18.

8. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M. Important flavonoids and their role as a therapeutic agent. Molecules. 2020 Nov 11;25(22):5243.

9. Umer SM, Shamim S, Khan KM, Saleem RS. Perplexing polyphenolics: The isolations, syntheses, reappraisals, and bioactivities of flavonoids, isoflavonoids, and neoflavonoids from 2016 to 2022. Life. 2023 Mar 9;13(3):736.

10. Shalaby MA, Moghazy FM, Shalaby HA, Nasr SM. Effect of methanolic extract of Balanites aegyptiaca fruits on enteral and parenteral stages of Trichinella spiralis in rats. Parasitology research. 2010 Jun;107:17-25.

11. Allam AF, Mostafa RA, Lotfy W, Farag HF, Fathi N, Moneer EA, Shehab AY. Therapeutic efficacy of mebendazole and artemisinin in different phases of trichinellosis: a comparative experimental study. Parasitology. 2021 Apr;148(5):630-5.

12. Mohamed SM, Taha AA, Abdel Hamed EF, Fawzy EM. Updated treatment modalities of Trichinellosis. The Egyptian Journal of Hospital Medicine. 2022 Oct 1;89(2):7680-3.

13. Otaki A, Furuta A, Asano K. Quercetin-induced enhancement of nasal epithelial cells' ability to produce Clara cell 10-kD protein in *vitro* and in *vivo*. Medicines. 2023 Apr 21;10(4):28.

14. García A, Barrera MG, Piccirilli G, Vasconi MD, Di Masso RJ, Leonardi D, Hinrichsen LI, Lamas MC. Novel albendazole formulations given during the intestinal phase of Trichinella spiralis infection reduce effectively parasitic muscle burden in mice. Parasitology International. 2013 Dec 1;62(6):568-70.

15. Nayak R. Histopathology techniques and its management. JP Medical Ltd; 2017 Dec 31.

16. Chan YH. Biostatistics 102: quantitative data–parametric & non-parametric tests. blood Press. 2003;140(24.08):79.

17. Chan YH. Biostatistics 103: qualitative data-tests of independence. Singapore Med J. 2003 Oct 1;44(10):498-503.

18. Chan YH. Biostatistics 104: correlational analysis. Singapore Med J. 2003 Dec 1;44(12):614-9.

19. Maged AA, Metwally KM, El-Menyawy HM, Hegab F, El-Wakil ES. Assessment of the potential prophylactic and therapeutic effects of kaempferol on experimental Trichinella spiralis infection. Journal of Helminthology. 2023 Jan;97:e36.

20. Huang H, Yao J, Liu K, Yang W, Wang G, Shi C, Jiang Y, Wang J, Kang Y, Wang D, Wang C. Sanguinarine has anthelmintic activity against the enteral and parenteral phases of trichinella infection in experimentally infected mice. Acta tropica. 2020 Jan 1;201:105226.

21. El-Melegy MA, Ghoneim NS, El-Dien NM, Rizk MS. Silver nano particles improve the therapeutic effect of mebendazole treatment during the muscular phase of experimental trichinellosis. Journal of American Science. 2019;15(5).

22. Petkova S, Dilcheva V, Gabev E. A Morphological Study on the Effect of Liposomally Administered Albendazole on the Trichinella spiralis Muscle Stage in Mice. Acta morphologica et anthropologica. 2014 Jun 6:89.

23. Siriyasatien P, Yingyourd P, Nuchprayoon S. Efficacy of albendazole against early and late stage of Trichinella spiralis infection in mice. Journal-Medical Association of Thailand. 2003 Jun;86(SUPP/2):S257-62.

24. Eid RK, Ashour DS, Essa EA, El Maghraby GM, Arafa MF. Chitosan coated nanostructured lipid carriers for enhanced in *vivo* efficacy of albendazole against Trichinella spiralis. Carbohydrate polymers. 2020 Mar 15;232:115826.

25. Chai JY, Jung BK, Hong SJ. Albendazole and mebendazole as anti-parasitic and anti-cancer agents: an update. The Korean journal of parasitology. 2021 Jun 30;59(3):189.

26. Fredericks J, Hill DE, Zarlenga DS, Fournet VM, Hawkins-Cooper DS, Urban Jr JF, Kramer M. * Inactivation of encysted muscle larvae of Trichinella spiralis in pigs using Mebendazole. Veterinary Parasitology. 2024 Apr 1;327:110140.

27. Attia RA, Mahmoud AE, Farrag HM, Makboul R, Mohamed ME, Ibraheim Z. Effect of myrrh and thyme on Trichinella spiralis enteral and parenteral phases with inducible nitric oxide expression in mice. Memórias do Instituto Oswaldo Cruz. 2015 Dec 11;110(8):1035-41.

28. El-Wakil ES, Abdelmaksoud HF, AbouShousha TS, Ghallab MM. Evaluation of Annona muricata (Graviola) leaves activity against experimental trichinellosis: In *vitro* and in *vivo* studies. Journal of Helminthology. 2021 Jan;95:e53.

29. El-Wakil ES, Khodear GA, Ahmed HE, Ibrahim GI, Hegab F, Abdo SM. Therapeutic efficacy of albendazole and berberine loaded on bovine serum albumin nanoparticles on intestinal and muscular phases of experimental trichinellosis. Acta Tropica. 2023 May 1;241:106896.

30. Solnier J, Martin L, Bhakta S, Bucar F. Flavonoids as novel efflux pump inhibitors and antimicrobials against both environmental and pathogenic intracellular mycobacterial species. Molecules. 2020 Feb 7;25(3):734.