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EFFECT OF KALOBIN (PELARGONIUM REINFORME/SIDOIDES EXTRACT) ON MURINE INTESTINAL *TRICHINELLA SPIRALIS*

By

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Abstract

Trichinellosis is a worldwide risky zoonotic nematode infecting people particularly in pig raising countries. The goal of this investigation was to evaluate the effectiveness of Kalobin (Pelargonium reniforme/sidoides) as a therapeutic and a prophylactic treatment of experimental intestinal *T. spiralis* infection. Consequently, one hundred Swiss albino male mice were divided into five groups of 20 each. GI: untreated, uninfected mice (negative control). GII: infected untreated mice (positive control). GIII: infected treated with Albendazole (ABZ) started three days after infection and continued for three successive days. GIV: infected and treated with Kalobin began 24 hours after infection and went on for 6 consecutive days since infection. GV: infected mice and Kalobin treated started 15 day before infection up to 6 successive days (pretreated group). Two hundred *T. spiralis* larvae were given orally to all groups except GI and evaluated parasitological, histopathological, and biochemical of the efficacy of treatment.The results showed that both GIII and GV caused substantial decrease in intestinal adults' count than other ones (94.4%, & 52.1% respectively). These results were supported by the enhancement of histology and biochemistry findings.

Keywords: Trichinellosis, Mice, Kalobin, Albendazole, GSH, IL-4, IL-10, MDA.

Introduction

Trchinella spiralis annually infected about 11 million people worldwide through consumption of raw or undercooked flesh of diseased livestock (Rózycki *et al*, 2022). In general, not less than 55 countries have reported zoonotic trichinosis (CDC, 2016).

In the Mediterranean and African regions, human trichinellosis was rare, and stems mostly from the religious practices and food habits but, sylvatic one was reported (Pozio, 1991). In Egypt, trichinosis was reported in man and animals (Morsy *et al*, 2022). Dyab *et al.* (2019) reported that prevalence of *T. spiralis* larvae in slaughtered pigs; from Governmental Basatin Slaughterhouse were 2/ 184 (1.08%). Mohammed *et al.* (2022) concluded that to have effective preventive and control measures for trichinosis pigs must not feed on garbage and preventing pigs slaughtering outside the slaughterhouses

Tissue damage in trichinellosis is a result of numerous variables in addition to the direct harm brought on by the parasite itself. The increased production of different stress markers, such as superoxide dismutase and malondialdehyde (MDA), indicates that the oxidative stress condition that comes along with *Trichinella* infection is one of the primary sources of this harm (Mido *et al,* 2012). Also, recruitment of inflammatory cells that, when activated, release an overabundance of nitrogen species, the reactive oxygen species (ROS) and the other free radicals (Chiumiento and Bruschi, 2009). T-helper cells are needed for immunological reaction against *T. spiralis* in the intestinal phase, Th1 and Th2 cells were stimulated during this process, with Th1 type initially predominating to achieve protection and parasite elimination (Ilic *et al,* 2021). IL-4, IL-5, IL-10, & IL-13 cytokines and IgE were secreted during this process (Bruschi and Chiumiento, 2012). Thus, IL4 and IL13 resulted in production of tumor necrosis factor (TNF) and interferon (INF-γ) to localize inflammation (Akiho *et al,* 2011). Nitric oxide is produced as a consequence of TNF release stimulating iNOS enzyme (NO). *T. spiralis* enteropathy is accelerated by inflammatory reaction

established by TNF-α and NO (Wink *et al*, 2010). Anti-inflammatory and antioxidants medications aid in the protection of humans (Kazemzadeh *et al*, 2014). However, widely used non-steroidal or steroidal medications have side effects restricted their use (Oray *et al*, 2016).

Thus, anti-parasitic therapies of plant or herbal extracts were tried. Myrrh and thyme mixtures were tested experimentally on murine *T. spiralis* (Attia *et al*, 2015). *Spinosa* leaf extract showed anti-trichinellosis efficiency (Yadav and Temjenmongla, 2012). The immunostimulant and effective remedy for respiratory infections Kalobin is a natural extract of *Pelargonium reniforme/sidoides* stems (Chuchalin *et al*, 2005). *P. reniforme/sidoides* contained significant quantities of calcium, silica, gallic acid, gallic acid methyl ester and tri- and tetra-oxygenated cumarine, among other useful substances (Alossaimi *et al*, 2022). Infections of the sinus, pharynx, respiratory system and tonsillo-pharyngitis and bronchitis were successfully treated with polyphenols compounds such as cumarin (Kayser *et al*, 2001). Besides, Gallic acid was impacted through the activation of the macrophage functions for generation of TNFα, iON, and IFN-γ, converting it as an anti-leishmanial therapy (Kolodziej *et al*, 2003).

The study aimed to evaluate the efficacy of Kalobin® as a therapeutic and a prophylactic treatment of experimental intestinal *T. spiralis* infection.

Materials and Methods

Clean laboratory bred male Swiss Albino mice about 25 ~ 30g and 6 to 8 weeks old were purchased from Theodor Bilharz Research Institute (TBRI). By repeated passage, *T. spiralis* strain was maintained & given about 200 larvae orally to each mouse.

Drugs:1- Albendazole®: Alzental suspension (EIPICO), a commercially available drug preparation was used in a dose of 20 mg/ml. 2- Kalobin®: A natural extract of *Pelargonium reniforme/sidoides* roots was purchased as an oral drops in a dose of 200µg/dl (Marcyrl Pharmaceutical Industries, El Obour City, Egypt).

Experimental design: A total of 100 Albi- no mice were classified into five groups of 20 mice each. GI: Neither infected nor treat- ed mice (negative control). GII: Infected, but untreated mice (positive control). GIII: Infected and Albendazole treated on the third post-infection day as 50mg/kg/day by an intra-esophageal gavage for three days (Li et al, 2012). GIV: Infected and Kalobin treated with dose of 200µg/dl started 24hr post-infection (P.I.) and continued for 6 successive days in a dose of 200µg/dl (Amer *et al*, 2006). GV: Infected mice and immune-stimulated with Kalobin at a dose of 200µg/dl started 15th day before infection and continued for 6 successive days (pretreated group). Ethical clearance: The experimental mice were kept in an appropriate animal house. The study was approved by the Scientific Research Ethical Committee Benha Faculty of Medicine (RC: 26-10-2022), which went with the Helsinki's Declarations (2008).

Histopathological study: Ten mice from every group were sacrificed on the 7th day PI and 1 ml intestinal samples were dissected out. Fixed samples were processed for paraffin sectioning (5µm) and (H & E) staining for histopathological examinations (Fischer et al, 2008).

Counting adult worms in the small intestine required cutting the remaining parts of each group into 2cm to be soaked in physiological saline at 37°C for 3 to 4 hours. Then the intestine was completely shaken in liquid and saline rinsed, the solution was centrifuged at 1500 rpm for five minutes and microscopically examined for the adults' counting in the sediment of each group (Wakelin and Lioyed, 1976)

For biochemical assays: The intestines of other ten mice of each group were dissected, rinsed in an ice- saline, sliced into numerous tiny fragments, weighed, homogenized in normal buffer phosphate solution before being centrifugation at 12,000g for 20 min at 4°C. The supernatant was subsequently maintained at 80°C until needed (lowry et al,1951)

For the evaluation of oxidant/anti-oxidant state in intestinal tissue homogenates, Biodiagnostic's commercial assays were used to measure the levels of MDA and reduced glutathione (GSH), both IL-4 and IL-10 were measured by ELISA (Ray-Biotech Inc., Peachtree Corners, Georgia, USA, and Chongqing Biospes Co., Chongqing, China, respectively), after the manufacturer's protocols and results were read on a micro-plate reader at 450nm with a correction wavelength marked at 570nm (Stat Fax®2100, Fisher Bioblock Scientific, Strasbourg, France)

Statistical analysis: Data were collected, tabulated, computerized and analyzed by the software SPSS (Statistical package for social science) version 25. Data were expressed as mean and standard deviation ±SD. Significant difference was evaluated by ANOVA and/or Kruskal-Wallis as indicated. P value <0.05 was considered significant.

Results

Histologically: By comparing GIII and GV to positive control, small intestine sections demonstrate a substantial reduction in level of inflammation and restored villi architecture. Specifically in lamina propria and core of the villi, GII demonstrated a dense inflammatory cellular infiltrate along with shortening, flatness, and proliferation of goblet cells. Also, core of the villi in the Kalobin-treated mice exhibited moderate inflammation.

Total *T. spiralis* adult count (TAC) in intestinal contents: TAC was performed on 7th day PI for all groups. A significant difference was between positive and negative controls. The mean TAC in GII (positive control) was 77.10±8.86. The TAC was significantly decreased in GIII (ABZ treated) compared with GII, which reached 4.40±2.22. There was insignificant decrease in TAC in GIV (Kalobin treated) compared with GII. As to the GV (Kalobin pretreated), there was significant decrease in the TAC in GV as compared with GII. As compared to the positive control, there was marked reduction in intestinal of adult worms' counts in all the treated groups.

Reduction rates in the intestinal phase were 94.4% in albendazole treated mice, 52.1% in pretreated Kalobin and 38.6% in Kalobin treated mice.

As to IL-4 concentrations positive and negative controls showed significant differences from one another. Levels of IL-4 in GII were 64.49±11.75. There was high significant decrease in GIII (ABZ treated) compared with GII. But, there was insignificant decrease in GIV compared with GII. But, comparing GII to GV showed a substantial decline. IL-10 levels in intestinal homogenates; there was a substantial disparity between the positive and negative controls.

In GII (positive control) the levels were 145.8±16.37. There was significant decrease in GIII (ABZ treated) compared with GII. Also, there was no significant decrease in GIV (Kalobin treated) compared with GII. The GV (Kalobin pretreated), there was significant decrease in GV compared with GII.

MDA levels in the intestinal homogenates were compared among all groups. Between positive and negative control, there were notable difference (1.45±0.28 & 3.21±0.42). There was a significant decrease in GIII compared with GII. There was insignificant de crease in GIV (infected Kalobin treated) compared with GII. GV showed significant decrease compared to GII.

Reduced glutathione in intestinal homogenates: Levels of reduced glutathione in positive and negative control showed a significant difference between one another. The levels of the reduced glutathione in GII were 1.21±0.11. There was a significant increase in GIII and insignificant increase in GIV as compared with GII. As to GV, there was significant increase in GV compared with GII.

Details were shown in tables (1 & 2) and figures (1, 2, 3, 4 & 5)

Table 1: Comparison of *T. spiralis* adult count in intestine among groups.

Groups	*T. spiralis* adult count in intestine	Reduction %	P-value
GI	0.0± 0.0	-	-
G II	77.10±8.86	-	-
GIII	4.40±2.22	94.4%	-
G IV	46.10±15.50	38.6%	0.010
G V	36.40±14.86	52.1%	0.048

P≤0.05 =significant,

Table 2: Levels of IL 4, IL 10, MDA and reduced glutathione among groups

Variations	GI	GII	GIII	GIV	G V	P value
IL 4 (pg/mg tissue)	29.49 ±3.93	64.49±11.75	40.08±7.38	52.75±9.09	49.44±11.76	p1<0.001 p2= 0.001 p3= 0.182 p4= 0.047
IL 10 (pg/mg tissue)	62.23± 15.15	145.81± 16.37	95.06±18.14	123.85±22.32	115.08±23.46	p1<0.001 p2<0.001 p3= 0.123 p4= 0.027
MDA (nmol/gm tissue)	1.45±0.28	3.21±0.42	2.35±0.64	2.89±0.28	2.52±0.32	p1<0.001 p2<0.001 p3= 0.429 p4= 0.005
Reduced glutathione (mg/ tissue)	5.09± 0.04	1.21±0.11	4.10± 0.33	2.39± 0.27	3.20± 0.36	p1<0.001 p2<0.001 p3= 0.109 p4= 0.003

P≤0.05 significant, P1: between GsII & I, p2: between GsII & III, P3: between GsII & IV, P4: between Gs II & V

Discussion

Albendazole therapy has been associated with transient and asymptomatic elevations in serum aminotransferase levels in up to 50% of patients treated for more than a few weeks. These abnormalities rapidly improve with stopping therapy which is rarely required (~4%). Albendazole has also been associated clinically apparent liver injury. The onset of injury has been within a few days to as long as 2 months of starting therapy.So it's crucial to develop new, secure, effective medications (Ben Fredj *et al*, 2014).To our understanding, no research has been done to determine how Kalobin affects *T. spiralis* infection.

In the present study, regarding the anti-parasitic impact showed a substantial drop in TAC in the infected mice and immune-stimulated with Kalobin (52.1%) compared to positive control. In agreement with the recorded observation, mice treated with herbal extract Alchinal, a complicated mixture made up of three ingredients (Echinacea purpura extract, *Allium sativum* extract and coca) harbored significantly fewer *T. spiralis* larvae (Bany *et al*, 2003). At this point in research, the exact process by which kalobin produces its anti-helminthic effect is unknown. However, it is thought that plant extracts may exert their effects through activation of antigen presenting cells as well as antibody production ((Amer et al, 2006).

In the present study, the histopathological examination of small intestines in infected mice and Albendazole treated and infected mice with Kalobin immune-stimulated showed significantly less inflammation than both the positive control group and Kalobin-treated group, as well as restored villi architecture. This agreed with Amer et al. (2006) in Egypt,they proved the immune-stimulating effect of Pelargonium reinforme/sidoides extract (Kalobin®) against Prohemistomum vivax Also, this agreed with Abdel Menaem et al. (2022) in Egypt who reported that Kalobin's immuno-potentiating effects on the treatment of schistosomiasis mansoni in vivo whether used alone or in conjunction with praziquantel. Gallic acid, the primary component of Pelargonium extract, was demonstrated by Kayser et al. (2001) to have an anti-leishmanial good effect. They noted that therapy with pelargonium extract resulted in the generation of TNF-ά, iNO, and INF-, which activated the macrophages.

Also, Tomex (garlic), according to Abou Hussien *et al.* (2022), has a potential in vitro antiparasitic action and may be an efficient alternate drug for *T. spiralis* adult worms and muscle larvae because it is a cost-effective, easily-accessible without the unpleasant smell of natural garlic plant. In the same time, *Nigella sativa* (black seeds), Ivermectin®, and Albendazole® were evaluated by Nada *et al.* (2018) for their ability to effectively treating mice infected with the different phases of *Trichinella spiralis.*

In the present study, IL-4 and IL-10 cytokines expressions were significantly down-regulated in GV compared to GII. But, between both GIII and GV, there was barely a change. By decreasing intestinal mastocytosis, which significantly contributed to villi injury and atrophy during *T. spiralis* infection, reduced IL-4 production may be beneficial in trichinellosis (Serna *et al,* 2006). More or less similar results were reported by Marshman *et al.* (2002); Sofronic-Milosavlijevic *et al.* (2013); Chen *et al.* (2013) and Ding *et al.* (2017), they showed that IL-4 mRNA is elevated in *T. spiralis* infected animals compared to uninfected ones. Goblet cell hypertrophy has been demonstrated to require the Th2 cytokines, particularly IL-4, which is a crucial aspect of intestinal nematode infection (Kuperman *et al,* 2005). According to Ding *et al.* (2017) TGF production, IL-4, IL-10, raised by more than three times when compared to the control ones. Additionally, while the production of IL-4 and IL-10 increased throughout the whole intestinal phase, IL-2 was down-regulated in early phases of infection. *T. spiralis* provided DCs with immunomodulatory capabilities and made them favor a Th2-polarized response (Stolley and Campbell, 2016). Free radicals and ROS are produced in large amounts during *T. spiralis* infection by both the parasite and the host as a result of inherent and learned immune reactions (Bruschi *et al,* 2003; Othman *et al,* 2016). The antioxidants are crucial to metabolism and aid in defending the host against oxidant-mediated negative impacts (Bruschi and Chiumiento, 2011). The positive control's small intestine had significantly higher levels of the oxidative stress indicators than the negative control group did. This agreed with many authors (Wojtkowiak-Giera *et al,* 2012; Blum *et al,* 2013; Kazemzadeh *et al,* 2014). The decline in MDA levels and the rise in GSH levels indicate that oxidative stress was reduced in GIII, GIV, & GV. Besides, the present data agreed with Hamed *et al.* (2022) who reported that curcumin's anti-inflammatory, antioxidant, and anti-angiogenic properties helped to alleviate trichinellosis and curcumin has potential as an adjunctive other antiparasitic drugs (Gabrashanska *et al,* 2019). Moreover, trichinellosis was successfully easily treated with antioxidant-agents like selenium and resveratrol (Elgendy *et al,* 2020).

Conclusion

The outcome results demonstrated how orlly taking Kalobin can guard against the pathological impacts of *T. spiralis* infection.This was significantly added by the anti-inflammatory and antioxidant effects of Kalobin. Accordingly, Kalobin may be helpful as an adjuvant in the therapy of trichinellosis and additional research on the use and exact mechanism of this adjuvant in both experimental animals and human deserves consideration.

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Explanation of figures

Fig.1: Section in small intestine of negative control (GI) showed normal intestinal villi.

Fig.2: Section in the small intestine of +ve control (GII) showed many scattered T.S. worms in lumen and within villi (red arrows) with distorted villous pattern (shorter and broader)(blue arrows) and increased goblet cell population (yellow arrow) as well as moderate infiltra- tion by mononuclear inflammatory cells (black arrow) (H&E stain, X100).

Fig. 3: Small intestinal section of Albendazole treated (GIII) of mice showed few T.S. worms (red arrow), nearly restorted villous pattern (blue arrows) and mild infiltration by mononuclear inflammatory cells (black arrow) (H&E stain, X100)
Fig.4: Small intestinal section (GIV) treated by Kalobin showed partially shortened villi (blue arrows), regenerating goblet cells yellow arrows) as well as moderate infiltration by mononuclear inflammatory cells (black arrow) (H&E stain, X100)
Fig.5: Small intestinal section of Kalobin pretreated (GV) showed partially retorted (blue arrows) and partially distorted villous pattern (red arrow) as well as mild mononuclear inflammatory cells.