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Possible improvement of praziquantel side effects by micronutrient supplementation

Objectives: Schistosomiasis is still one of the most important parasitic diseases in Egypt. Treatment of schistosomiasis depends almost exclusively on praziquantel (PZQ). Although it was regarded as safe generally, the comprehensive use of praziquantel induced several common adverse reactions. **Methods:** This study aimed to clarify the modulatory effect of micronutrients (in the form of oral syrup named Vitamount) on low (250 mg/Kg) and high dose (500 mg/Kg) of PZQ in reducing praziquantel side effects on one hand and increasing its efficacy on the other hand. *In vitro* and *in vivo* study of a new synthetic compound: 2-(3-Benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-4-yl)-3- *p*- tolylthiazolidin-4-one (BPPT) was evaluated as anti schistosomal drug.
Results: Data obtained from the present study showed that group taking PZQ (500 mg/kg) plus Vitamount achieved the best reduction in worm burden and ova count in addition to achieving the best improvement in hepatic antioxidant enzymes when compared with all other treatments. Such combination restored the alterations in all parameters exerted by *Schistosoma* infection towards normal levels. **Conclusion:** It was previously known that PZQ (500 mg/kg) is effective in eradicating worm burden and ova count. The present study proved that co-administration of Vitamount guard against PZQ side effects by enhancing the antioxidant status.

Keywords: *Schistosoma mansoni*, Praziquantel, oxidative stress, BPPT.

INTRODUCTION

Schistosomiasis remains a significant health burden for many parts of the world, particularly where health resources are most limited (King, 2010).Of the 239 million people with active *Schistosoma* infection in 2009 (Barros *et al*., 2009), 85% lived in sub-Saharan Africa, where an estimated 150,000 deaths/year were attributable to schistosomiasis (Van der Werf and De Vlas, 2001; King *et al*., 2011). Schistosomiasis tops all the endemic parasitic diseases world-wide particularly in Egypt (Shenawy, Soliman and Reyad, 2008).

Chemotherapy is the mainstay of schistosomiasis control and is carried out largely through the use of praziquantel (PZQ) (Sayed *et al*., 2008). It is considered the current drug of choice against schistosomiasis. PZQ, a pyrazinoisoquinoline anthelmintic compound, is active against all schistosome species and is also effective against other trematode and cestode infection (Greenberg, 2005). Because of these advantages over other chemotherapeutics, PZQ has, in recent years, become effectively the only anti*schistosoma*l commercially available drug (Hagan *et al*., 2004). Tens of millions receive annual treatments of PZQ (Sayed *et al*., 2008; Fenwick A. 2006). PZQ administration can result in many significant side effects including dizziness, meningism headache, sleepiness, fatigue, vertigo, abdominal pain, cramps, nausea, vomiting, diarrhoea, bloody stools, low back pain and urticaria/rash (José Carlos *et al*., 2010), seizures and transient increase in liver enzymes (Beck et al., 2001; Ali, 2011). Besides, it was reported that praziquantel induce hemorrhage in the lung tissue of the host (Flisser and Mclaren 1989).

Micronutrients are those vitamins and minerals required in minuscule amounts, these substances enable the body to produce enzymes, hormones and other substances essential for proper growth and development. *Schistosoma* infection may enhance host malnutrition which may influence parasite establishment, maturation, survival and expulsion. The relationship between malnutrition and parasite infection can be synergistic, which means that a preexisting malnutrition lowers host resistance to infection or increases duration or severity of the infection. The opposite, or antagonistic type of interaction, results in an infectious process that is less severe in a mal-nourished host than in a well-nourished one (Olsen, Nawiri and Friis, 2000).

The aim of the present study was to improve the efficacy of PZQ through some micronutrients supplementation on one hand and to reduce its side effect on the other hand. This was done by studying the effects of PZQ and micronutrients either alone or in combination on several parasitological and biochemical parameters in clean and infected mice with *Schistosoma mansoni*. As a trial to introduce a new anti*schistosoma*l drug, the efficacy of BPPT was studied.

MATERIALS AND METHODS

Chemicals: Chemicals used were purchased from Adwic, Fluka, Sigma Aldrich Co. (St. Louis, MO, USA), EIPICO and Amoun (Egypt).

Animals: Laboratory-bred female Swiss albino mice, each weighing 18-20 g, were used in the study. They were maintained in conditioned rooms at 21̊ C on sterile water ad libitum and balanced dry food.

Cercariae: *Schistosoma mansoni* cercariae suspension was obtained from SBSP/TBRI. Infection was performed by subcutaneous injection of 80 *S. mansoni* cercariae for each mouse (Stirewalt and Dorsey, 1974).

This study was conducted in accordance with legal ethical guidelines of the Ethical Committee of the Federal Legislation and National Institute of Health Guidelines in USA and approved by the Ethics Committee of the National Research Center in Egypt.

Drug regimen

1. **PZQ:** It was kindly gifted from Egyptian International Pharmaceutical Industries Co. (EIPICO). The drug was freshly prepared and orally administered to mice using a stainless steel oral cannula. Two doses of PZQ were tested (250 and 500 mg/kg b.w.) for two consecutive days.
2. **Vitamount:** A multi micronutrient syrup (Amoun Pharmaceutical Company**)** containing (Vitamin A 1800 i.u. , Vitamin E 30 i.u. , Vitamin C 60 mg , Vitamin B1 2.5 mg , Vitamin B2 1.7 mg , Vitamin B3 20 mg , Vitamin B6 2 mg , Vitamin B12 6 µg , Vitamin D 400 i.u. , biotin 300 µg , calcium pantothenate 10 mg , iodine 150 µg iron 9 mg , zinc 3 mg , manganese 25 mg and chromium 25 µg per 15 ml) was administered in a dose of 2.6 ml/kg body weight (Olsen, Nawiri and Friis, 2000; Paget and Barnes, 1964).
3. **New compound under exploration:** 2-(3-Benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-4-yl)-3-*p*- tolylthiazolidin-4-one (BPPT), is a new compound prepared in our laboratories (NRC laboratories, Therapeutical Chemistry Department). The tested dose was 50 mg/kg body weight for two consecutive days (75 % of LD_{50}).

***In vitro* assessment of the possible antischistosomal effect of BPPT :** To determine the LC_{50} of BPPT , a stock solution of 2-(3-Benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-4-yl)-3- *p*- tolylthiazolidin-4-one (BPPT) was prepared in dimethyl sulfoxide (DMSO) , diluted with RPMI media to produce test solutions of different concentrations ranging from 10-100 µg/ml. Three replicates were used for each concentration, 12 worms, males and females equally represented, were placed in each vial using sterilized tissue forceps. Incubation was maintained at 37ºC. Positive (praziquantel, 0.1 µg/ml) and negative (DMSO) controls were similarly performed. Examination for worm viability was done after 24 h using a stereomicroscope. Worms showing no signs of motility for 1 min were considered dead.

***In vivo* determination of LD_{50} for 2-(3-Benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-4-yl)-3-*p*-tolylthiazolidin-4-one (BPPT)**

Median lethal dose (LD_{50}) was determined orally according to the method of Behrens and Karber (1970).

Experimental design: Animals were divided into ten groups. Twenty mice were allocated for each infected group and 12 mice for each clean non infected group. Groups 1-5 were infected mice that were kept for 6 weeks after infection. Groups 6-10 represented clean non infected mice. Group 1 served as control infected , groups 2-5 were orally administered praziquantel (500 mg/kg), praziquantel (500 mg/kg) + Vitamount , praziquantel (250 mg/kg) + Vitamount and BPPT, respectively. Vitamount was given 4 weeks before infection then mice were infected and Vitamount supplementation continued for another 6 weeks (groups 3, 4 only) then treatments (PZQ or BPPT) was given for 2 consecutive days. Mice were sacrificed after 2 weeks. The clean non infected groups (6-10) were administered their respective treatments simultaneously alongside with the infected treated groups.

Sampling: At the end of the experimental period, animals were sacrificed. Liver perfusion of 5 mice from each infected group was separately performed for worm counting. At the end of perfusion, the liver was removed and divided into 3 fragments for ova count. Liver of the remaining animals from each group were separately homogenized using an electrical homogenizer and the resulting homogenates were centrifuged at 3000 r.p.m. for 15 min in a refrigerated centrifuge and the

supernatants were stored at – 20°C to be used for enzymatic assays.

Parasitological parameters

Worm count: Worms were recovered by hepatic perfusion and percent of reduction in worm number was calculated by the method of Tendler *et al*., (1986). **Ova count:** The liver was dissected and divided into 3 fragments for ova count according to the method of Cheever and Andeson (1971).

Determination of hepatic antioxidant enzymes:

Determination of glutathione reductase (GR) was performed according to the method of Carlberg and Mannervik (Carlberg, Mannervik B. 1975). Thioredoxin reductase (Thrxs), was assessed according to the method of Holmgren and Björsnstedt (1995). Determination of glutathione peroxidase (GPX) and glutathione-S-transferase were performed according to the method of Lawrence and Burk (1976) and Habig, Pabst and Jacoby (1974), respectively.

Statistical analysis:

The results were presented as mean ± standard deviation (S.D.) for 9 mice in each group. Results were analyzed statistically by one way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences, version 9) software. When an overall significance was indicated by the F value, differences were considered statistically significant at $p < 0.05$.

RESULTS

Parasitological studies:

***In vitro* studies**

In vitro schistosomicidal activity of 2-(3-Benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-4-yl)-3-*p*-tolylthiazolidin-4-one (BPPT) on *Schistosoma mansoni* adult worms

Different concentrations of BPPT and its corresponding % mortality were represented in Tab.I. It showed that LC_{50} of BPPT in worms is 30 µg/ml.

***In vivo* studies**

Effect of different treatments on worm load and ova count in infected mice

In vivo studies showed that co-administration of Vitamount with PZQ 500 mg/kg achieved the best reduction in worm burden followed by PZQ 500 mg/kg alone reaching 99.5% and 94.7%, respectively (Tab.II). Results of worm load were supported by results of ova count where simultaneous administration of Vitamount with PZQ 500 mg/kg succeeded in recording the best ova count reduction (83.5%), in comparison with PZQ 500 mg/kg alone that achieved only 77.1% reduction in ova count.

Biochemical studies:

Effect of different treatments in clean and infected mice on antioxidant enzyme activities in liver homogenates

A significant decrease in hepatic antioxidant enzyme activities due to infection was measured. The best recovery in different antioxidant enzyme activities among all groups was apparent in the group treated with PZQ 500 mg/Kg + Vitamount. Supplementation of Vitamount with PZQ did not succeed in decreasing the used dose of PZQ as shown in the group treated with PZQ 250 mg/Kg + Vitamount. Although BPPT significantly increased antioxidant enzyme activities but the enhancement was very low when compared with PZQ 500 mg/Kg.

In clean mice, the co-administration of Vitamount with either doses of PZQ (PZQ 500 mg/kg or PZQ 250 mg/Kg) exerted no significant change in all antioxidant enzyme activities when compared with non treated group. On the other hand, PZQ alone induced significant decrease in all antioxidant parameters measured followed by BPPT when compared with non treated group (Tab.III).

DISCUSSION

Given the extend of schistosomiasis problem , and the fact that treatments relies on a single drug , praziquantel , which raises issues with respect to resistance and some side effects , there is a need for more studies on PZQ . In this work we first use the supplementation of some micronutrients in form of Vitamount to improve the side effects of PZQ on one hand and to compensate the decrease in micronutrients due to *Schistosoma* infection on the other hand. Second, we tried to find out a new drug for treating schistosomiasis.

The present work showed that PZQ 500 mg/kg body weight, administered to infected mice, induced reduction of total worm burden (94.7%) and decrease in ova count (77%), which is supported by Morsy (2009) who determined a reduction in both worm burden (99%) and egg count (63.69%) when using the same dose and duration of PZQ. Hendawy *et al*., (2010) confirmed that PZQ (500 mg/kg for two consecutive days) caused a marked reduction in worm burden reaching 95.6%, the oogram pattern after PZQ treatment showed a complete disappearance of all immature ova from the wall of the intestine. Mantawy, Ali and Rizk (2011) found that treatment with praziquantel (500 mg/kg b.w.) on two

successive days after 45 days of infection resulted in significant reduction in worm burden (95.8%) accompanied with significant increase in percentage of dead ova (87.3%) and a decrease in the percentage of mature ova stages (12.7%), reduction in hepatic and intestinal oogram by 90.7% and 93.8%, respectively. Damage caused by PZQ increases exposure of antigens on the worm surface, particularly over male worm tubercles and this in turn renders the worms more susceptible to antibody attack. This drug-induced antigen exposure is assumed to account for the synergistic effect between PZQ and host antibodies in killing worms in vivo (Doenhoff, 2010).

It was reported that *Schistosoma* infection induces a decrease in some micronutrients. Saleh and Shehata (1979) measured a decrease in thiamine, pantothenic acid, and niacin levels due to schistosomiasis. Mikhail and Mansour (1982) and Kaestel *et al*., (1999) reported that infected patients show subnormal levels of plasma vitamin A, retinol binding protein, prealbumin and zinc when compared to the control group. Berhe *et al*., (2007) found that serum retinol concentrations were inversely related to intensity of *S. mansoni* infection. Serum retinol may transiently be low due to reduced production of retinol binding protein, which is often observed as part of acute-phase response to infection (Thurnham, McCabe, Northrop-Clewes, Nestel, 2003). Therefore, there was a great need of micronutrients supplementation during *Schistosoma* infection to compensate their decrease. Maraini *et al*., (2009) reported that daily used multivitamin-mineral supplements formulated at about RDI levels can significantly raise the plasma levels of many of the nutrients included in such supplements.

In the current work, co-administration of multi-micronutrient (Vitamount) to PZQ 500 mg/kg induced more reduction of total worm burden (99.5%) and more decrease in ova count (83%) than that achieved from either PZQ 250 mg/kg + Vitamount or PZQ 500 mg/kg alone.

Treating the clean mice with PZQ negatively affect all measured antioxidant parameters. The addition of Vitamount improved these negative effects which is an evidence of the positive effect of using it.

The data obtained in the present study showed significant decrease in antioxidant enzyme activities including GR, GPX, Thrxs and GST in infected mice. These results coincide with those of Farrag and Faddah (1998). They found that glutathione peroxidase and glutathione -S- transferase activities were decreased in infected mice compared to non infected animals. Also, Sheweita *et al*. (1998 and 2010) found that the activity of GST was decreased in human and mice infected with *S. mansoni*. Similar results were reported by Gharib, Abd-Allah, Dessein and De-Reggi (1999). They measured a decrease in GPX activity in livers of mice infected with *S. mansoni*. Also, Mantawy, Ali and Rizk (2011) found significant decrease in GPX activity due to *Schistosoma* infection. The decrease in antioxidant enzyme activities can be referred to schistosomiasis which induces different symptoms in the host such as anemia and inflammation. The increase in free radicals during schistosomiasis is mainly attributed to these two symptoms as well as their induction during phagocytosis (Martin *et al*., 2004). Other proposed mechanism for the decrease in antioxidant enzyme activities seems, at least in part, to be a specific effect of protein deficiency that can alter the turnover of some enzymes. The mentioned hypothesis is supported by Gharib, Abd-Allah, Dessein and De-Reggi (1999). They attributed the decrease in antioxidant enzyme activities during *Schistosoma* infection to several mechanisms that may account for such discrepancy such as increased cytotoxicity with H_2O_2 which is produced as a result of inhibition of glutathione reductase that keeps glutathione in its reduced form and decreased translation of mRNA into protein. This again is supported by El-Rigal and Hetta (2006) and El-Ansary, Ahmed and Aly (2007). They reported a decrease in protein content in infected host. They attributed it to mRNA degradation. Huang and Fwu (1992) demonstrated that low protein not only retards animal growth but also changes the metabolism via modification of the hormonal profile. As a result, turnover of some enzyme protein could be specifically altered. For example, the liver microsomal detoxification enzyme activities and inducibility were shown to be lowered by low protein levels (Kato, Tard and Yoshida, 1980).

Another factor for the decrease in antioxidant enzyme activities may be due to the depletion of the cofactors, in this case, NADPH which can be attributed to the inhibition of glucose-6-phosphate dehydrogenase (G6PDH). GPx and GR act in consort, with G6PDH supplying reducing equivalent (NADPH). NADPH is needed for GR activity which in turn maintains adequate concentration of GSH required for GPx activity. Also, there is a decrease in glutathione synthetase activity required for GSH synthesis (Chitra and Devi, 2008).

Unavailability of GSH can also cause a reduction in the activity of GPx and GST (Chitra and Devi, 2008).

Data obtained from the present work showed that PZQ 500 mg/kg induced significant increase in antioxidant enzyme activities when compared with infected mice. Similar findings were reported by Sheweita et al. (2010) who showed that Praziquantel treatment of *S. mansoni* infected mice succeeded in normalization of glutathione reductase and glutathione –S- transferase. Mantawy, Ali and Rizk (2011) also supported our findings where they found that glutathione peroxidase level showed significant increase after PZQ treatment. The amelioration in antioxidant status may be attributed to reduction in worm load as a consequence of PZQ treatment.

Best improvement achieved in our study was after Vitamount co administration with PZQ 500 mg/kg. All biomarkers of oxidative stress were changed in the treated groups indicating the efficacy and the protective effect of the antioxidant supplementations against the oxidative insult derived from infection. Despite that some reviews and studies point out that antioxidant intervention with micronutrients in different human diseases failed to show positive effects (Halliwell, Rafter and Jenner, 2005; Hercberg, 2006; Block *et al*., 2006; Brigelius-Flohé, 2009; Halliwell, 2009), in the present study the antioxidant therapy with Vitamount (which includes Vitamin A, Vitamin E, Vitamin C, zinc and chromium) was effective in improving the liver antioxidant parameters associated with *Schistosoma* infection. Therefore, such nutritional intervention might be recommended for subjects exposed to S. infection. Our mentioned assumption was supported by several authors where Castenmiller *et al*. (1999), reported an increase in GR activity after 3 weeks of dietary intervention with carotenoids which is known to deactivate singlet oxygen. Esen and Tekeli (2009) suggest that trivalent chromium supplementation increase antioxidant enzyme activities. Moreover, Filho *et al*., (2010) revealed that after supplementation with vitamins C and E all biomarkers of oxidative stress were improved in human exposed to occupational airborne contamination from coal mining extraction and incineration of hospital residues. The interception of the peroxyl radical (RO2•) by α-tocopherol results in the formation of the tocopheroxyl radical, which is regenerated back to α-tocopherol by ascorbate or reduced glutathione. In fact, ascorbate can act either directly in cellular membranes by blocking the beginning of the lipoperoxidation process, or indirectly by regeneration of tocopheroxyl radical to vitamin E (Traber and Atkinson, 2007; Zingg, 2007). This indicates the efficacy of the protective effect of antioxidant supplementations against the oxidative insult derived from schistosomiasis.

Combining PZQ 250 mg/kg with Vitamount is not sufficient to achieve significant amelioration in all parameters measured in infected mice. It can be attributed to the fact that PZQ 250 mg/kg is not a sufficient curative dose. The remained viable worms induce oxidative damage in the host.

Results of the current study demonstrated that, *in vitro* assay of different concentrations of BPPT revealed its schistosomicidal activity where 100, 80, µg/ml and 30 µg/ml succedded in achieving 100%, 91.6% and 50 % mortality respectively. BPPT consists of 3 rings: Benzofuran, Pyrazol and Thiazolidinone where the last is the most important ring regarding the anthelmintic activity. Several studies reported that different thiazolidine derivatives are considered as potent anthelminthic compounds (Silva, SilvaGoes, De Lima, Souza Mala, 2003). Taha and Soliman (2007) documented tegumental alterations in *S. mansoni* worms induced by a thiazolidine containing chemical compound where administration of different doses to infected mice resulted in extensive loss of spines and the tubercles lost their normal shape and fused together forming bubble-like lesion in some areas.

Due to the promising *in vitro* results of BPPT, *in vivo* studies were performed. *In vivo* BPPT administration induced reduction in worm load (81.5%) and ova count (75.66%) in infected mice but this reduction was not efficient as that achieved by PZQ (500 mg/kg) alone or PZQ (500 mg/kg) + Vitamount.

Regarding antioxidant parameters, BPPT showed significant improvement in all antioxidant enzyme activities; however, its efficacy is lower when compared with either PZQ (500 mg/kg) alone or PZQ (500 mg/kg) with Vitamount.

CONCLUSION

The curative dose of Praziquantel (500 mg/kg) was able to eradicate schistosomiasis and should not be decreased even in the presence of other supplements. The present study supported the co-administration of micronutrients to the treatment protocol beside PZQ. The addition of Vitamount to PZQ counteracted some of the side effect of the PZQ on one hand. On the other hand, Vitamount is

very effective in attenuating the oxidative insult associated with *Schistosoma mansoni* infection. In addition; the compound under investigation (BPPT) did not show the expected *in vivo* efficacy when compared with either its *in vitro* one or that of PZQ.

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Table I. Effect of different concentrations of BPPT on *S. mansoni* adult worm viability.

Concentration(µg/ml)	Number of total worms	Number of dead worms	% Mortality
100	12	12	100%
80	12	11	91.6%
60	12	11	91.6%
50	12	10	83.3%
40	12	9	75%
30	12	6	50%
20	12	1	8.3%
10	12	0	0%

Table II. Effect of different treatments on worm load in infected mice.

Animal groups	**Worm burden**			**Total worm burden**	**Ova count**
	Male	Female	**Couples**		
Control infected	11.6 ± 2.07	2.8 ± 0.83	12.8 ± 1.92	40 ± 15.81	23208 ± 4290.84
PZQ 500 mg/kg	0.7 ± 0.89	0.4 ± 0.54	0.5 ± 0.85	2.1 ± 0.7 [a]	5320 ±1505.65[a]
(%Reduction)	93.96 %	85.71 %	96.09 %	94.7 %	77.07**%**
PZQ 500 mg/kg + V.	0.2 ± 0.44	0	0	0.2 [a]	3939.2 ± 885.25 [a]
(%Reduction)	98.72%	100 %	100 %	99.5%	83.02%
PZQ 250 mg/kg + V.	2.2 ± 0.83	1.2 ± 0.83	2.8 ± 0.83	9 ± 0.00 [a]	6909.8 ± 1077.4 [a]
(%Reduction)	81.03 %	57.14 %	78.12 %	77.5 %	70.22**%**
BPPT(0.14 mg/kg)	1.8 ± 0.83	0.8 ± 0.83	2.4 ± 1.14	7.4 ± 2.79 [a]	5648.4 ± 1153.19 [a]
(%Reduction)	84.4 %	71.42 %	81.25 %	81.5 %	75.66**%**

Values are means ± S.D, each group consists of 5 mice, [a] $P < 0.05$ compared to control infected group. PZQ = Praziquantel, BPPT = 2-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-3- p-tolylthiazolidin-4-one, V. = Vitamount

Table III. Effect of different treatments in clean and infected mice on antioxidant enzyme activities in liver homogenates.

Groups / Parameters	Clean control	Control infected	Infected PZQ 500 mg/kg+V	Infected PZQ 250 mg/kg+V	Infected PZQ 500 mg/kg	Infected BPPT	Clean PZQ 500 mg/kg+V	Clean PZQ 250 mg/kg+V	Clean PZQ 500 mg/kg	Clean BPPT
GR	86 ± 5.63	26.1 ±5.27 [a]	74.7 ± 4.7 [b]	46.4 ± 5.1 [ab]	65.1 ± 5.32 [ab]	45.1 ± 4.6 [ab]	77.1 ± 5.25 [b]	84.6 ± 5.54 [b]	68.66 ± 4.71 [ab]	55.5 ± 5.68 [ab]
Thrxs	47 ±5.09	13 ± 3.9 [a]	37 ± 4.5 [ab]	29.6 ± 3.8 [ab]	33.2 ±4.57 [ab]	35.7 ± 3.9 [ab]	42.3 ± 3.8 [b]	45.5 ± 4.77 [b]	39.3 ± 4.58 [ab]	44.6 ± 3.27 [b]
GPX	3.6 ±0.46	2.25 ±0.4 [a]	3.15 ± 0.33 [b]	2.65 ± 0.43 [ab]	2.88 ± 0.38 [ab]	2.86 ± 0.33 [ab]	3.42 ± 0.49 [b]	3.6 ± 0.45 [b]	2.98 ± 0.26 [ab]	2.96 ± 0.29 [ab]
GST	0.4 ±0.03	0.13±0.04 [a]	0.3 ± 0.04 [ab]	0.2 ± 0.036 [ab]	0.3 0.045 [ab]	0.24 ± 0.03 [ab]	0.313 ± 0.04 [ab]	0.334 ± 0.035 [ab]	0.257 ± 0.027 [ab]	0.25 ± 0.032 [ab]

Values are means ± S.D, each group consists of 9 mice, [a] $P < 0.05$ compared to clean control group and [b] $P < 0.05$ compared to control infected group. PZQ = Praziquantel, BPPT = 2-(3-Benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-4-yl)-3- *p*- tolylthiazolidin-4-one, V = vitamount.