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Potentiation of efficacy of ivermectin in *Haemonchus contortus* by P- glycoprotein modulators and/or inhibitors

Om Prakash, S Gomathinayagam, TJ Harikrishnan, M Raman, V Pandiyan and Samapika Sahoo

Abstract
The *Haemonchus contortus (H. contortus),* parasitic gastrointestinal nematode is of important concern for sheep and goat industry. Commonly known as the barber's pole worm, abomasual worm, wire worm *H. contortus* can affect a great damage to a flock or herd of sheep and goat within a short period of time. Presentexperiment was conducted to evaluate the rational compounds to potentiate the efficacy of ivermectine in resistant *H. contortus* sheep and goat.Larval migration inhibition assay (LMIA) were carried out to assess the potentiation effect of ivermectin resistant *H. contortus*. In LMIA, 42% of the samples were ivermectin resistant with LM_{50} value of 0.149. Curcumin, Quercetin, Kaempferol, Phloretin, Verapamil and Loperamide were selected for this study and *In vitro* reversal of ivermectin resistance in *H. contortus* with co-incubation compounds were carried out. The potentenciation efficacy of ivermectin was noticed with highest concentration of chemo sensitizers with LM_{50} of resistant larvae dropping from 0.14902, to 0.0005, 0.0031, 0.0015, 0.0089, 0.0041 and 0.0046 for Verapamil, Loperamide, Quercetin, Kaempferol, Phloretin and Curcumin respectively.

Keywords: P-glycoprotein, haemonchus, ivermectin. sheep and goat

1. Introduction
Due to growing human population and its increasing opulence, the demand of livestock products such as milk, meat, wool and fiber is expected double during first half of the 21st century [1]. Haemonchosis, the disease associated with *Haemonchus contortus (H. contortus)* infections, may be acute, hyper acute or chronic with minor clinical signs, resulting in death. The use of anthelmintics in intensive farming has followed swiftly by the emergence of anthelmintic resistance (AR) and AR is now an emerging phenomenon in parasitic nematodes of sheep, goats, horses, and cattle [2]. P-glycoproteins (Pgps) are efflux transporters that belong to the ATP binding cassette (ABC) superfamily which actively transport compounds, including drugs, across membranes [3]. The primary function of Pgp is to protect the organism by actively pumping toxic substances out of its cells [4-6].
P-glycoproteins have been identified in *H. contortus* and the full cDNA sequence has been obtained [7]. The mechanism believed to be associated with anthelmintic resistance in *H. contortus* is the overexpression of Pgp. Both benzimidazole and ivermectin-resistant strains of *H. contortus* have been found to possess Pgp alleles in higher frequency than susceptible strains. A role for P-glycoprotein (P-gp) drug efflux pumps in ML resistance in *H. contortus* and other parasitic nematodes was reviewed [8]. The expression of P-gps has been increased in IVM resistant isolates [7, 9, 10]. Third generation Pgp inhibitors including tariquidar, zosuquidar and elacridar increased the efficacy of IVM, levamisole (LEV) and thiabendazole [11]. *In silico* analysis of the binding of anthelmintics *to Caenorhabditis elegans* P-glycoprotein [12]. Recently, interaction of Curcumin with the involvement of higher number of amino acids as compared to thymoquinone using i*n silico* molecular docking had conducted and concluded that curcumin could be more effective in inhibiting the antioxidant enzymes of *F. gigantic* [13].
Even though several herbal and synthetic chemicals were found to alter the resistance to BZ/IVM in *H. contortus,* some of these compounds are quite toxic and cannot be used in food animals. Hence, it is imperative that safe ecofriendly compounds, whether synthetic or herbal with strong affinity to interact with Pgp, need to be identified to potentiate the efficacy of the fewer anthelmintics available for animals.

2. Materials and Methods

2.1 Collection of Parasites and Harvesting of Eggs

Abomasal contents of small ruminants sheep and goat with live worms *H. contortus* were collected in normal saline from slaughter house, Perambur, Chennai, India during period of January, 2013 to March, 2015. In the laboratory, the contents with live worms were transferred to a plastic tray containing normal saline. The adult male and female *Haemonchus contortus* (*H. contortus*) worms were separated by gross morphology and washed twice in the normal saline. The female worms were incubated in normal saline at 37 ^{0}C for 2 hours for the release of eggs naturally and other worms were discarded. After incubation, the normal saline was collected in centrifuge tubes and centrifuged at 2000rpm for 5min to sediment the eggs. The supernatant was poured off and the sediment was examined for the presence of eggs. The concentration of the eggs was adjusted to 50 eggs per 20µl of normal saline and aliquoted.

2.2 Harvesting of larvae

The dung pellets from sheep and goat were collected from the slaughter house, Perambur, Chennai and checked for parasite status. The pellets were homogenized and further autoclaved [14], to kill larvae, if any, present. Coprocultures were made as per method described [15]. The third stage larvae were collected into a 15 ml centrifuge tube using a sterile Pasteur pipette and was centrifuged at 1000 rpm for 15 minutes. The supernatant was discarded and 1 ml of the sediment was collected separately into another tube. About 100 µl of the sediment was taken to a slide and the number of larvae was counted under a binocular stereo zoom microscope (Olympus SZ40, Japan). The larvae were used for LMIA.

2.3 Reagents and Chemicals Preparation

2.3.1 Preparation of ivermectin (IVM) stock solution (2mM)

To prepare a stock solution of 10^{-2}M, 8.71mg of Pure Ivermectin (Sigma-I-8898, USA) was weighed and dissolved in 1 ml of dimethyl sulfoxide (DMSO, BP231-1, Fisher Scientific, USA) and mixed thoroughly. Using the stock solution, a suitable range of working solutions was prepared.

2.3.2 Preparation of IVM working solution

A wide range of working solutions of ivermectin with final concentrations of 10-3, 10-4, 10-5 and 10-6M were prepared by serially diluting the IVM stock solution.

2.3.3 Preparation of stock solutions of Pgp modulators/inhibitors

Pure verapamil (Sigma Aldrich -V-4629, USA) 16.84mg was weighed and dissolved in 10ml of distilled water and mixed thoroughly. 200µL of this solution was added in each well to reach a final concentration of 343µM of verapamil [16]. Loperamide (Sigma Aldrich- L4762-5G, USA, 5.13mg), Curcumin (Sigma Aldrich- C7727-500MG, USA, 3.68 mg), Phloretin (Sigma Aldrich- P7912-100MG, USA, 2.74 mg),Quercetin Hydrochloride (Sigma Aldrich- Q3001-50MG, USA 2.74 mg) and Kaempferol(Sigma Aldrich- K0133-50MG, USA, 3.68 mg) were weighed and dissolved in 1 mL of Dimethyl Sulfoxide (DMSO, BP231-1, Fisher Scientific, USA) separately to prepare a stock solution of 10-2M of each compound.

2.3.4 Preparation of working solutions of LPM, Curcumin, Phloretin, Qurecetin, and Kaempferol

Using the stock solutions, a suitable range, 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-6}M of working solutions of each compound was prepared by serially diluting the stock solution as done earlier

2.4 Larval migration inhibition assay (LMIA)

The assay was based on the paralysing property of the drug ivermectin and the ability of resistant larvae to migrate through sieves at higher concentration of IVM drug than those of susceptible strains. It was carried out as per method described [17].

2.5 Potentiation of ivermectin by LMIA

The procedure for Potentiation of ivermectin by LMIA was done as per the method described [17] with minor modifications

3. Results

3.1 Larval migration inhibition assay (LMIA)

In the present study larval migration inhibition assay were conduted to asses of IVM resistance and susceptible of *H. contortus* in small ruminants. LMIA was based on the ability of larvae to migrate through 25µm mesh at a concentration greater than 10^{-2}M of ivermectin. In LMIA, larvae migrated through mesh size of 25µm exposed to a concentration greater than 10^{-2}M of ivermectin were considered resistant. In present study, 41.0 per cent were found IVM resistant. The standard error for the mean percentage of larval migration for IVM resistant and susceptible population of *H. contortus* at different concentrations of IVM. The mean migration percentage of resistant and susceptible populations of *H. contortus* were statistical analysed by one way ANOVA (Duncan) and found to be level of significance $P<0.001$ between and within groups. Using probit analysis, Mean LM_{50} value for Ivermectin resistance and susceptible population were 0.1492 and 0.0001 respectively LM_{50} values were calculated. The resistance factor was 1492. **(Table.1)**.

3.2 Potentiation of IVM by Pgp Modulators/inhibitors through LMIA

The LMIAs were conducted to see the Potentiation of IVM by Pgp modulators/inhibitors as similar to ivermectin resistance and susceptible of *H. contortus*. The standard error for the mean percentage of larva migrated through sieve were calculated when treated with combination of IVM and P-glycoprtien modulators i.e. Verapamil, Loperamide, Quercetin, Kaempferol, Phloretin and Curcumin and statistically analysed by one way ANOVA (Duncan) and found to be level of significance $P<0.001$ between and within groups, when IVM resistant larvae were incubated with minimum concentrations of different plant compounds / chemicals as (Table 2). Using probite analysis LM_{50} values are calculated for Verapamil (Fig.1), Loperamide (Fig2) Quercetin (Fig3), Kaempferol (Fig4) Phloretin (Fig5),and Curcumin (Fig6). Present study showed that all the P-gp modulators/inhibitors were able to partially potentiate the ivermectin efficacy dose depended.

4. Discussion

4.1 Larval migration inhibition assay (LMIA)

Larval migration inhibition assay was successfully performed to assess the ivermectin resistance. The migration assay is a rapid and cost effective tool for the determination of the

effects of drugs that paralyze nematodes [18]. Many authors utilized mesh sizes of 20, 25, 28 and 30µ to find out the migrating larvae [19, 20], But mesh size of 25µ was recommended by Freie Universitat of Berlin especially for *H. contortus*. Hence, 25µ mesh was used in this study.

LMIA or modified migration assay had been used to evaluate the resistance or susceptibility to IVM in Kenya [21]; in Australia [22]; in Belgium [23]; in Puerto Rico [24]. The method adopted in this study was based [17] with modification who evaluated the ability of the larval migration inhibition test (LMIT) to detect ivermectin resistance in cattle and sheep nematodes like *Ostertagia ostertagi*, *Cooperia oncophora*, and *H. contortus* in Germany. IV M resistance was confirmed when larvae migrated through at the highest concentration of 10^{-2}M.In this study, those larvae migrated at the concentration of 10^{-2}M were considered as resistant larvae.

In present study, forty one percent of pooled larval samples were found to be ivermectin resistant through LMIA. The work reported here contributes with further evidence on the high degree of resistance to IVM in *H. contortu s*under field conditions. The percentage of larval migration at different concentration of IVM (10^{-2}M to 10^{-6}M) was 57.86, 64.53, 72.93, 79.93 and 84.73 and LC_{50} was 0.142. in Tamil Naduobserved that infective larvae had 22.0, 18.0 and 25.0 per cent migration even at the maximum concentration (0.4 µg) of levamisole and the LC_{50} values were 0.0156, 0.0286 and 0.0227 µg / ml, respectively[25].LM_{50} values were 0.707, 0.437 and 0.377 µg / ml of levamisole in three farms[26]. the ivermectin resistance in *H. contortus* of sheep and goat and found that the mean percentage of larval migration at different concentrations (10^{-2}M to10^{-8}M) was 32.1, 38.8, 47.8, 68.4, 83.3, 85.6 and 87.4 indicating resistance [27]. The findings of this study are in conformity with earlier reports and confirm that there is emergence of IVM resistance also in *H. contortus* in Tamil Nadu.

Larval migration test performed to evaluate the efficacy of buck wheat *Fagopyrum esculentum* Moench (ETM) as anthelmintic against GI Parasites of sheep and found that the ETM inhibited 17.66% of larvae migration in the concentration of 5 mg mL-^{1}[28]. All these reports proved the usefulness of this test for finding out sensitivity or resistance of any drug that has the potential to paralyses the parasites. Earlier reports from Tamil Nadu used this test for evaluating LEV and IVM resistance. In this study, IVM resistance was successfully detected in forty one percent of the samples tested (N=290).

On the contrary, micromotility test was more sensitive for quantitatively measuring the degree of resistance between susceptible and resistant isolates of *H. contortus* compared larval migration inhibition test[29].The RFs for this test for IVM and eprinomectin ranged from 1.00to 108.05 and from 3.87 to 32.32, respectively. But they suggested that LMIT might be a superior tool to monitor resistance to Moxidectin.

4.2 Potentiation of IVM by Pgp Modulators/inhibitors through LMIA

Reversals of IVM resistance by these compounds by LMIA were calculated. The least concentration of reversal compounds that have capacity to inhibit the fifty percentage of larval migration through mesh is as follows; VRP (2mM); Loperamide (10^{-3}M); Quercetin (10^{-3}M); Kaempferol (10^{-3}M); Phloretin (10^{-3}M) and Curcumin (10^{-3}M) respectively. There is reversal to susceptibility as the LM_{50} values had gone below the discriminating level of 10^{-2}M after addition of these compounds. There *in vitro* activity of IVM against larvae of *H. contortus* was increased from 3-10 folds after co incubation with Pgp modulators/ inhibitors. Higher exposure to IVM obtained after co-incubation with P-gp modulators/ inhibitors accounted for a higher efficacy i.e., larval migration inhibition in resistant *H. contortus*.

In this study, we observed the *in vitro* (LMIA) potentiation of IVM efficacy against the resistant isolate of *H. contortus*, when this ML was associated with different P-gp modulators/inhibitors. These compounds at the given concentration inhibited Pgp mediated resistance by preventing the efflux of the drug or potentiating the toxicity of IVM resulting in the inhibition of larval migration However, this potentiation did not reach maximum efficacy (100%) in any of the drug combinations evaluated here. The partial or absent phenotypic reversion of IVM resistance by some drugs evaluated in this study can be explained by their low P-gp affinity or the occurrence of other mechanisms of resistance, such as changes in enzyme systems and/or cellular receptors.

Multidrug resistance transporters (MDR) actively drive out many types of xenobiotics from cells including drugs such as the MLs [30]).This theoretical assumption on drug–transporter interaction was assessed using *in vitro* assays and clinical efficacy studies in laboratory animals.

Many researchers proved that the *in vitro* co- incubation or *in vivo*co- administration of Pgp modulators with ivermectin resulted in the increased efficacy of ivermectin. The efficacy of both IVM and moxidectin against resistant *H. contortus* strains was increased in the presence of the P-gp modulators verapamil and CL347099 [31, 32]and cyclosporine A, [33].The presence of p-gp inhibitor Verapamil, there was enhanced susceptibility of the cattle nematode *C. oncophora* to Ivermectin *in vitro* as measured using a larval developmental test and larval migration inhibition test [20]. *In vitro* and *in vivo* the efficacy of both ivermectin and moxidectin against resistant *Cooperia* spp. in cattle tended to increase after their co-administration with loperamide (LPM) as a P-gp modulator [34]. An increased IVM efficacy from 53.1% to 94.3%*in vitro* with Verapamil and they demonstrated 36.02% efficacy with IVM + VRP compared to the 7.75% of IVM alone [35]. The association of IVM with Cyclosporine-A, Ceftriaxone, Dexamethasone, Diminazene aceturate, Quercetin, trifluoperazine, Verapamil, or vinblastine on the efficacy of IVM and found that the efficacy was increased [36]. Increased toxicity of IVM to resistant larvae (up to 5.7-fold decrease in ivermectin LC_{50}) when co-administered with crizotinib, and rendered the resistant larvae equally or more sensitive to IVM than the susceptible isolate [37]. Thus, many compounds and chemicals have been tried for interaction with Pgps to increase the toxicity of IVM.

In this study six compounds have been tried successfully to increase the toxicity of IVM and it has been demonstrated in LMIA that all the compounds were able to potentiate the effect of IVM. The association of IVM with some modulating drugs evaluated here significantly reduced IVM LM_{50} values. LM_{50} levels decreased 3.5 folds in Curcumin to almost 300 folds in case of Verapamil. This is in agreement with the findings that the *in vitro* activity of IVM against *Cooperia* spp. was increased between 10- and 100-fold after the co-incubation with the P-gp modulator [38]. The effect of P-gp modulators verapamil, Loperamide, Quercetin, Kaempferol, Phloretin and Curcumin on the sensitivity of susceptible and resistant populations of *H. contortus.* Perusal of the reports published earlier indicates that Kaempferol and Phloretin

Curcumin have not been tested in anthelmintic resistance. However Verapamil and Loperamide and Quercetin had been used. The results of the study are coherent with many of the earlier findings.

Some researchers have conducted studies and found the potentiating effect of Pgp modulators/inhibitorsi.e. herb rosemary [39]; piperine [40]; Biochanin [41]; Flavonoids Kaempferol and Quercetin [42, 43]; Loperamide [44]; the stemofoline derivatives including OH-A1, NH-B6 and NH-D6 showed commitment efficiency to increase sensitivity to doxorubicin, vinblastine and paclitaxel in KB-V1 cells and increase sensitivity to doxorubicin, and paclitaxel in K562/Adr cells whereas the effects have not been seen in their parental sensitive cancer cell lines (KB-3-1)[45]. Pharmacological interactions between drugs without clinical toxicity may be a future alternative to control resistant nematodes and retard the development of resistance to anthelmintic drugs such as IVM in the field as opined by (Ballent *et al*., 2006) [46]. The exposing cells to p-gp inhibitors led to increase of reactive oxygen and higher synthesis of ATP This ultimately resulted in cell death [47]. Potential side effects of the modulating agents, their relatively short persistence and changes to the pattern of tissue residues are among issues to be addressed before any practical applications can be advised [48].

In conclusion, the use of P-gp modulators Verapamil, Loperamide Quercetin Kaempferol, Phloretin and Curcuminat certain concentrations associated with IVM showed *in vitro* capacity to potentiate IVM efficacy against an IVM-resistant field isolate of *H. contortus*, as shown by the inhibition of larval motility. The *in vitro* potentiation of IVM observed in this study may be the step to discover new modulating drugs that completely (100%) restore IVM efficacy against gastrointestinal nematodes of small ruminants under field conditions. *In vitro* studies using this model, specific assays on P-gp activity, and the investigation of several other possibly unknown mechanisms of resistance are required to further the knowledge about the interaction between IVM and modulating drugs. Additionally, the pharmacodynamics, pharmacokinetics, and clinical safety of these modulating drugs should be studied *in vivo* [36].

Table 1: Mean Percentage of Larval Migration in Ivmr and Ivms Population of *H. contortus* (N= 290)

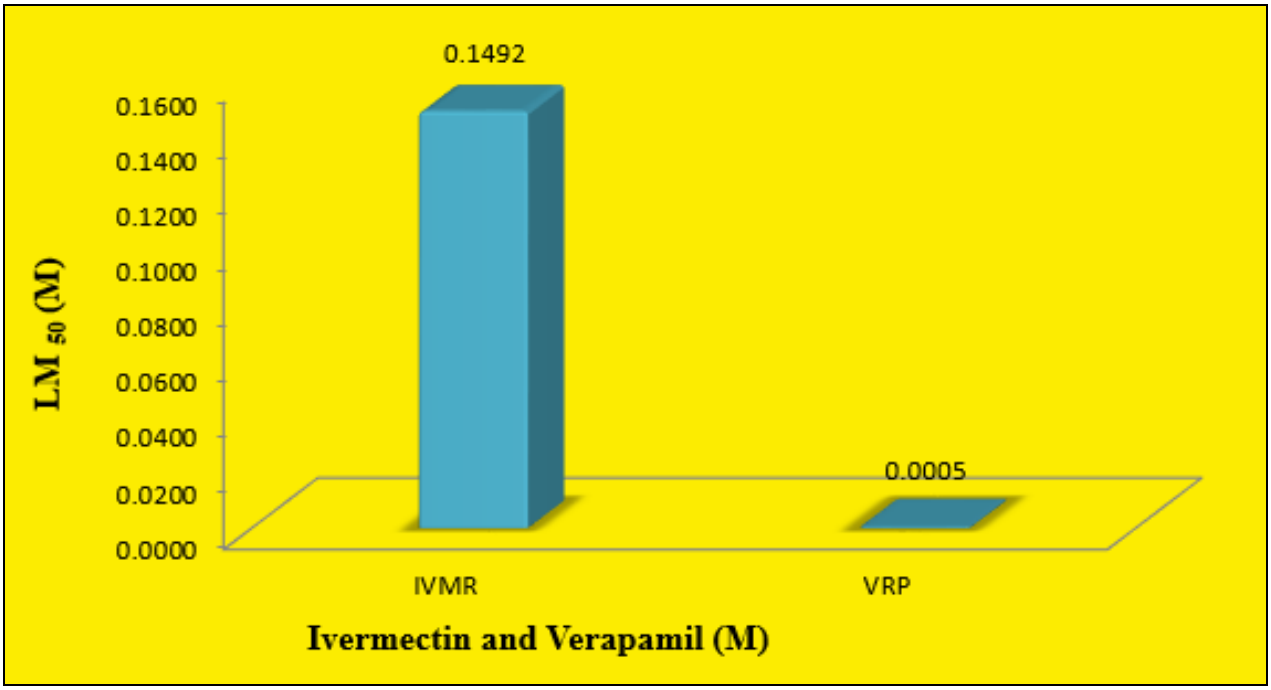
H. contortus	SEMof Larval migration percentage at different IVM concentration						LM_{50}	RF
	CONTROL DMSO	10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M		
IVMR	90.06±0.21	57.86±1.23	64.53±3.21	72.93±1.43	79.93±3.21	84.73±0.91	0.1492	1492
IVMS	87.06±1.21	16.46±2.32	22.26±1.21	36.73±3.23	50.66±3.02	69.13±0.6	0.0001	

IVMR -Ivermectin Resistance; IVMS- Ivermectin Susceptible; SEM- Standard error for the mean
LM_{50}-50 Percent Migration inhibition; RF- Resistant Factor
Level of significance $P<0.001$ between and within groups

Table 2: Mean Percentage of Migrated Larvae in Ivermectin or In Combination with Pgp Modulators/Inhibitors

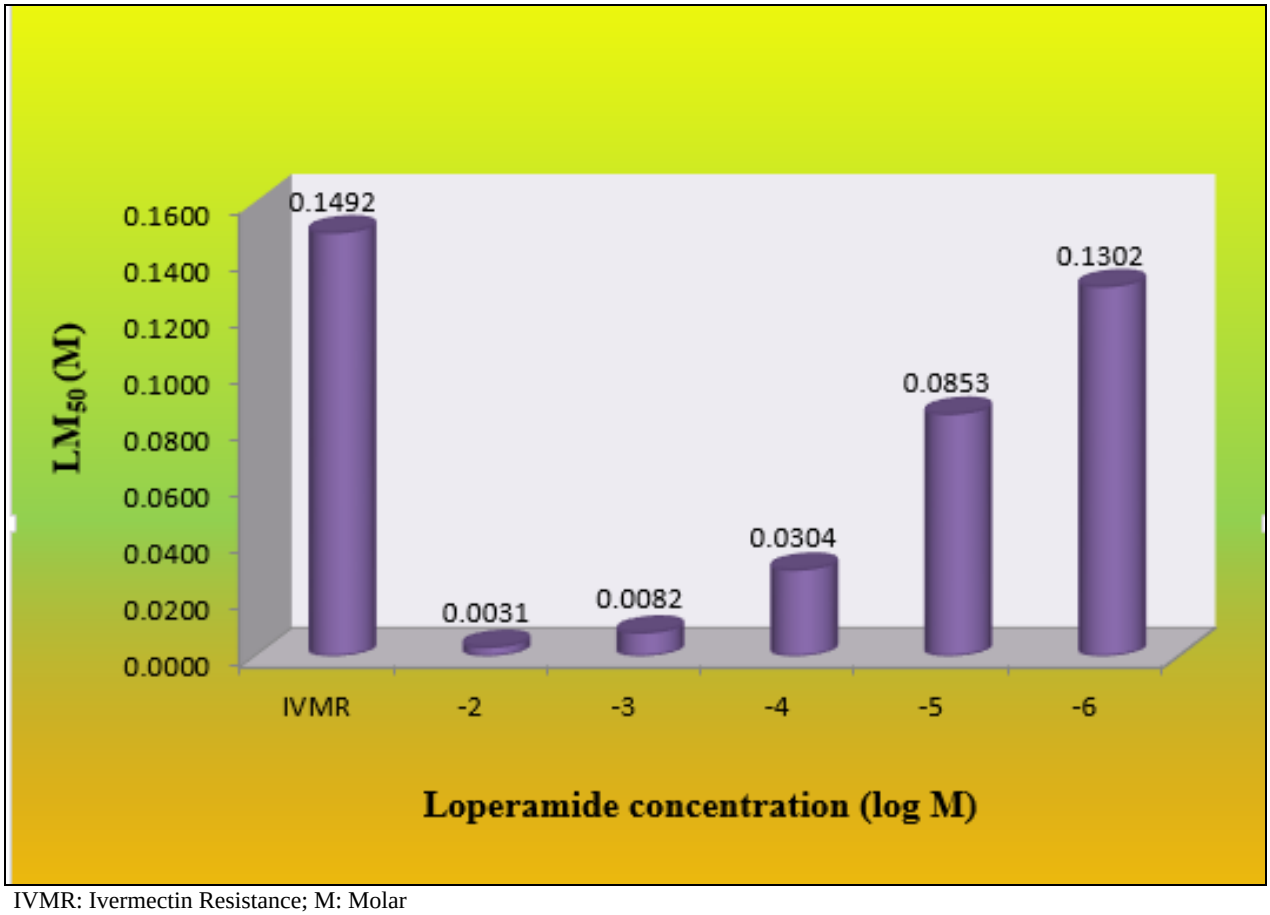
Parameters	SEM of migrated larvae at different IVM concentration and Pgp modulators/inhibitors					
	Control	10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M
IVMS	87.06±1.21(DMSO)	16.46±2.32	22.26±1.21	36.73±3.23	50.66±3.02	69.13±0.6
IVMR	90.06±0.21 (DMSO)	57.86±1.23	64.53±3.21	72.93±1.43	79.93±3.21	84.73±0.91
VRP	80.93±0.32(VRP)	27.38±4.02	35.53±0.23	47.62±2.11	59.13±2.54	68.93±1.87
L10^{-2} M	82.82±0.43 (L)	47.21±2.05	58.42±1.32	62.43±0.54	74.21±0.56	80.25±1.43
L10^{-3} M	87.41±1.2 (L)	49.32±0.35	61.61±3.21	70.21±032	77.62±2.61	85.31±0.67
L10^{-4} M	84.82±0.78 (L)	51.19±0.45	58.67±1.45	66.43±1.51	71.8±1.32	80.31±0.84
L10^{-5} M	86.89±0.67 (L)	62.89±2.79	68.58±1.34	72.89±3.11	79.81±1.45	81.09±0.57
L10^{-6}M	86.64±0.42 (L)	64.85±0.61	70.91±0.87	75.67±0.43	80.12±1.24	83.53±1.05
Q10^{-2} M	84.64±0.23 (Q)	32.42±4.02	44.71±0.43	58.32±0.77	66.21±0.71	79.52±1.48
Q10^{-3} M	82.31±0.21 (Q)	44.82±0.84	51.42±0.47	66.32±1.43	74.42±1.56	83.92±0.34
Q10^{-4} M	80.21±0.23 (Q)	49.62±0.21	60.32±1.23	69.32±2.52	75.32±0.43	82.01±0.67
Q10^{-5} M	86.89±1.23 (Q)	56.82±0.87	62.78±0.55	69.98±1.3	72.82±3.21	82.81±0.4
Q10^{-6} M	86.34±4.21 (Q)	61.34±1.67	67.32±2.43	72.2±0.98	76.45±0.43	84.14±0.78
K10^{-2} M	85.42±4.3 (K)	44.62±2.12	55.32±0.32	59.81±1.32	69.81±1.31	80.21±1.21
K10^{-3} M	80.23±3.21(K)	48.78±2.65	60.22±0.32	65.32±	71.52±0.55	79.02±0.87
K10^{-4} M	81.32±3.2 (K)	55.61±1.89	61.63±0.45	67.66±2.43	78.63±4.43	83.11±1.42
K10^{-5} M	83.41±1.89 (K)	60.83±1.43	66.61±1.48	72.11±3.21	80.32±1.42	82.82±0.43
K10^{-6} M	83.21±0.23 (K)	60.21±0.56	65.43±4.23	70.32±0.21	78.32±0.56	82.12±3.21
P10^{-2} M	79.19±2.10(P)	37.52±3.21	51.54±1.24	62.21±0.83	72.87±0.21	78.89±1.23
P10^{-3} M	83.32±6.31(P)	47.42±0.87	58.83±1.34	64.48±1.34	79.84±0.76	80.23±0.43
P10^{-4} M	85.32±0.42(P)	51.89±1.34	63.78±1.12	64.42±1.52	73.81±0.53	79.94±0.32
P10^{-5} M	83.41±1.89(P)	60.83±1.43	66.61±1.48	72.11±3.21	80.32±1.42	82.82±0.43
P10^{-6}M	84.33±3.20(P)	62.94±0.65	66.32±0.61	68.43±0.56	74.84±0.32	80.21±1.34
C10^{-2} M	81.93±1.34 (C)	39.13±3.02	49.13±0.98	63.53±1.32	71.93±7.17	79.66±0.89
C10^{-3}M	83.22±2.31 (C)	42.53±1.73	52.86±1.54	65.86±3.43	75.26±0.43	80.65±1.45
C10^{-4}M	84.66±3.2 (C)	51.66±0.67	59.06±0.32	68.66±1.34	73.66±0.53	80.73±1.32
C10^{-5}M	86.33±1.32 (C)	54.8±0.33	61.82±0.67	73.66±2.12	77.4±0.65	83.83±3.21
C10^{-6} M	84.66±0.31 (C)	59.66±0.76	64.26±3.21	68.66±0.53	75.53±0.54	84.46±0.6

*IVMS- ivermectin susceptible; IVMR- ivermectin resistance; VRP- Verapamil, L- Loperamide, Q-Quercetin, K- Kaempferol, P- Pholretin, C- Curcumin, Level of significance $P<0.001$; SEM- Standard error of the mean; M- Molar



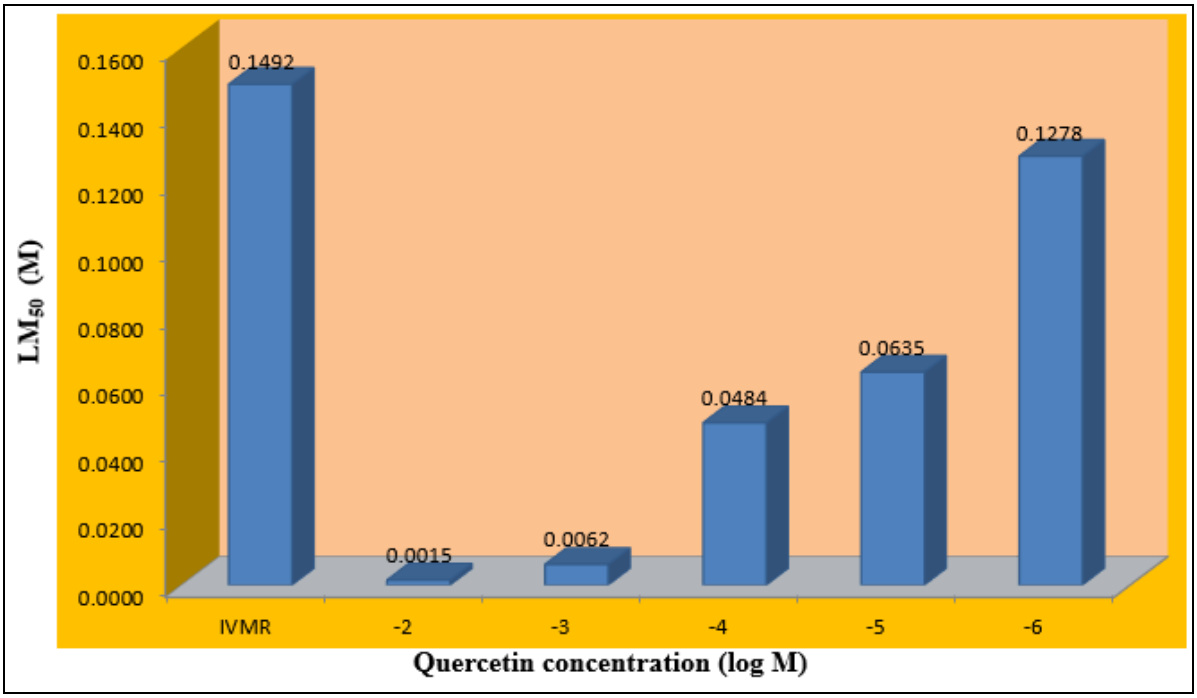
IVMR: Ivermectin; VRP: Verapamil; M: Molar

Fig 1: Mean Lm_{50} Values After Co- Incubation with Verapamil



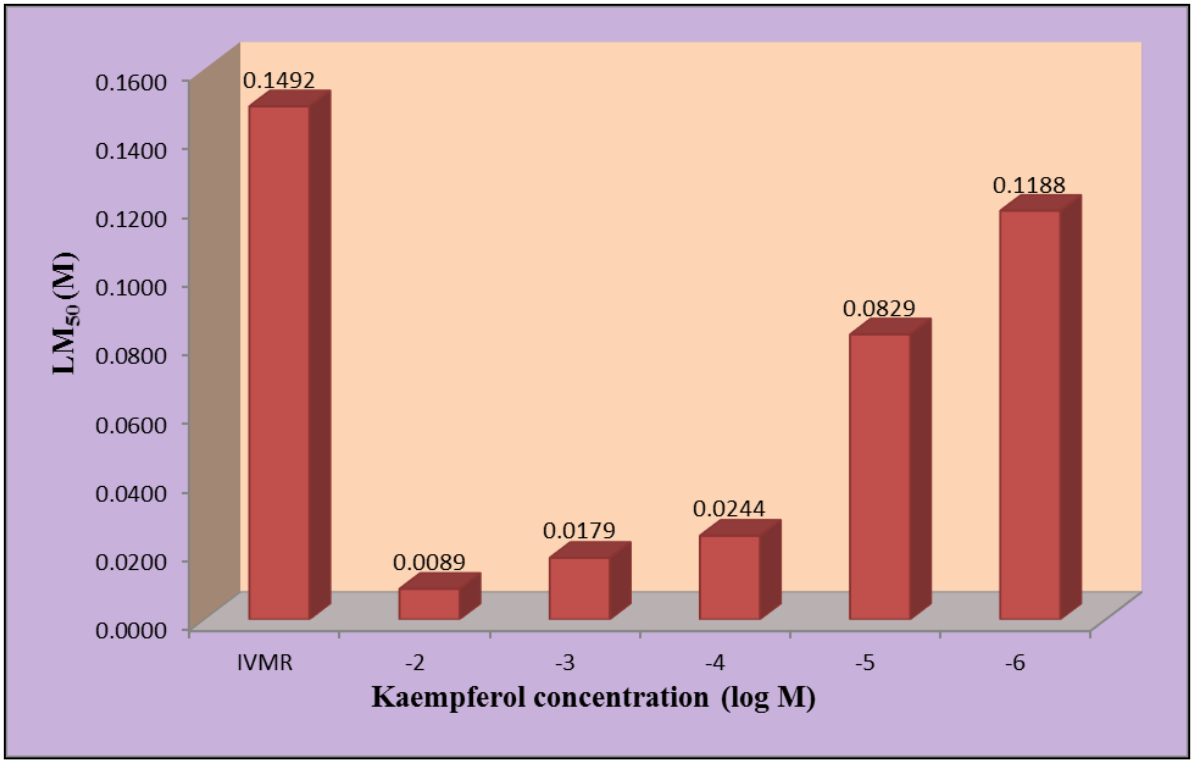
IVMR: Ivermectin Resistance; M: Molar

Fig 2: Mean Lm_{50} Values After Co- Incubation with Loperamide



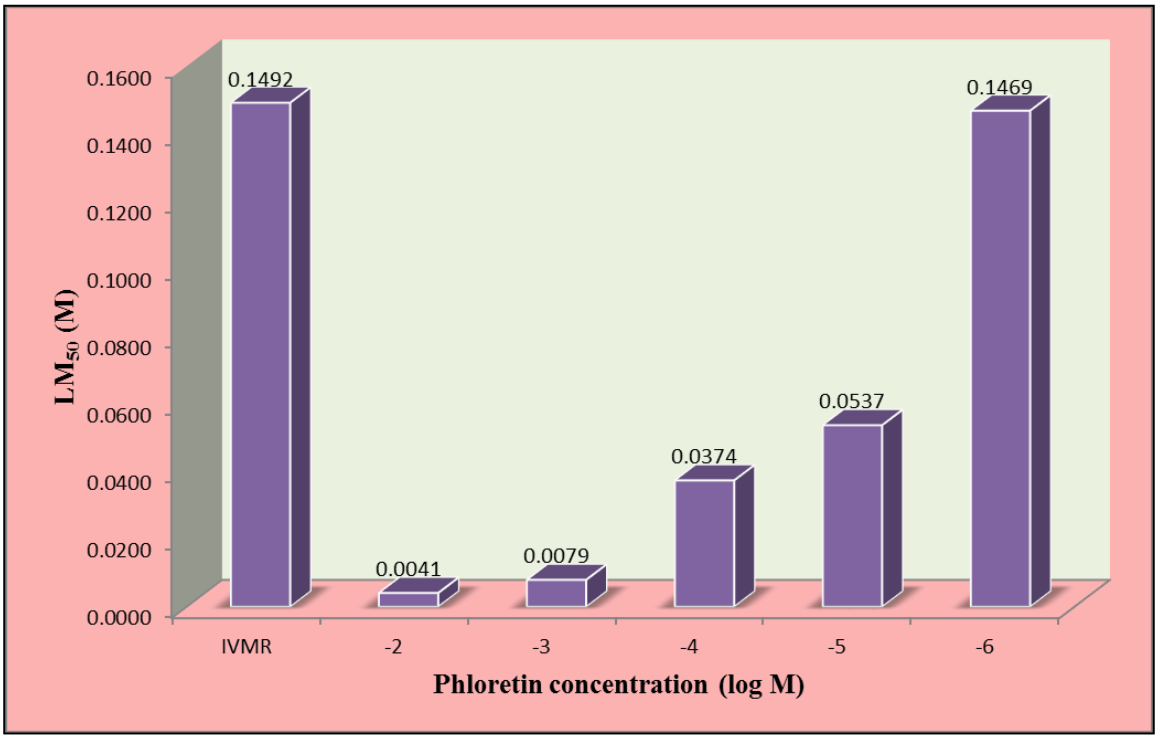
IVMR: Ivermectin Resistance; M: Molar

Fig 3: Mean Lm_{50} Values After Co- Incubation with Quercetin



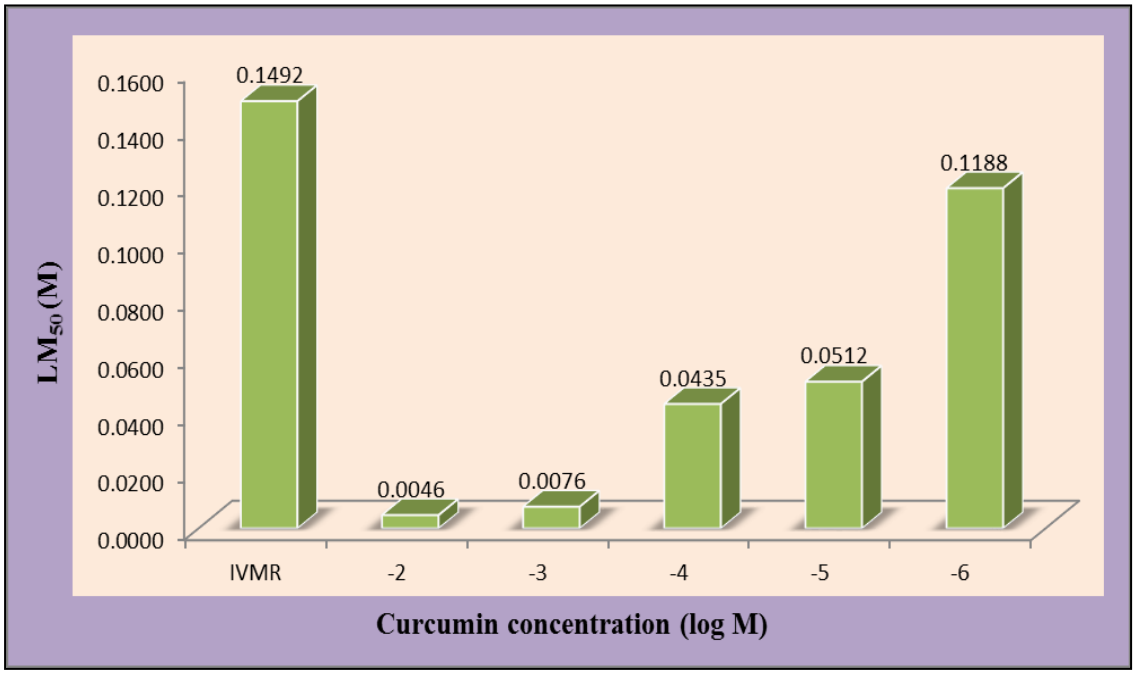
IVMR: Ivermectin Resistance; M: Molar

Fig 4: Mean Lm_{50} Values After Co- Incubation with Kaempferol



IVMR: Ivermectin Resistance; M: Molar

Fig 5: Mean Lm_{50} Values After Co- Incubation with Phloretin



IVMR: Ivermectin Resistance; M: Mola

Fig 6: Mean Lm_{50} Values After Co- Incubation with Curcumin

5. Conclusion

A study was conducted to evaluate the rational compounds to potentiate the IVM in *H. contortus*. Larval migration inhibition assay (LMIA) were carried out to assess the Ivermectin resistance. In LMIA, 42% of the samples were ivermectin resistant with LM_{50} value of 0.149. Reversal of ivermectin resistance was noticed with highest concentration of chemo sensitizers with LM_{50} of resistant larvae dropping from 0.14902 to 0.0005, 0.0031, 0.0015, 0.0089, 0.0041 and 0.0046 for Verapamil, Loperamide, Quercetin, Kaempferol, Phoretin and Curcumin respectively.

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