

**Review Article****Open Access****A review of the anti-viral effects of ivermectin***¹Adegboro, B., ²Lawani, O. A., ³Oriaifo, S. E., and ⁴Abayomi, S. A.Departments of ¹Medical Microbiology & Immunology, and ³Clinical Pharmacology, Nile University, Abuja, Nigeria²Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria⁴Department of Medical Microbiology and Parasitology, LAUTECH Teaching Hospital, Ogbomosho, Nigeria*Correspondence to: boazadegboro@gmail.com**Abstract:**

Ivermectin is an avermectin which is a group of pentacyclic sixteen-membered lactone (macrocylic lactone disaccharide) derived from the soil bacterium *Streptomyces avermitilis*. It is a semi-synthetic broad-spectrum anti-helminthic, anti-viral and anti-cancer agent. It has a wide safety margin with low adverse effects when it is used orally. It has, however, so far only been approved by the Food and Drug Administration (FDA) as a broad spectrum anti-parasitic agent. Because ivermectin also has broad activities as an anti-viral agent, we herein review its pharmacokinetic and pharmacodynamic activities, as well as the *in vitro* and *in vivo* studies conducted on the drug. It is hoped that this work will pave way for ivermectin being seriously considered as an addition to the drugs available for the management of patients with COVID-19.

Keywords: ivermectin; pharmacokinetics; pharmacodynamics; broad-spectrum anti-viral; COVID-19

Received Oct 31, 2020; Revised Apr 20, 2021; Accepted Apr 21, 2021

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Un examen des effets antiviraux de l'ivermectine*¹Adegboro, B., ²Lawani, O. A., ³Oriaifo, S. E., et ⁴Abayomi, S. A.Départements de ¹Microbiologie médicale et d'immunologie, et ³Clinique de pharmacologie,
Université du Nil, Abuja, Nigéria²Département de microbiologie médicale et de parasitologie, Hôpital universitaire d'Ilorin, Ilorin, Nigéria⁴Département de microbiologie médicale et de parasitologie, Hôpital universitaire de LAUTECH, Ogbomosho, Nigéria*Correspondance à: boazadegboro@gmail.com**Abstrait:**

L'ivermectine est une avermectine qui est un groupe de lactone pentacyclique à seize membres (lactone disaccharide macrocyclique) dérivée de la bactérie du sol *Streptomyces avermitilis*. C'est un anthelminthique semi-synthétique à large spectre, antiviral et anticancéreux. Il a une large marge de sécurité avec de faibles effets indésirables lorsqu'il est utilisé par voie orale. Cependant, jusqu'à présent, il n'a été approuvé que par la Administration des aliments et des médicaments (FDA) en tant qu'agent antiparasitaire à large spectre. Étant donné que l'ivermectine a également de vastes activités en tant qu'agent antiviral, nous passons ici en revue ses activités pharmacocinétique et pharmacodynamique, ainsi que les études *in vitro* et *in vivo* menées sur le médicament. On espère que ce travail ouvrira la voie à l'ivermectine qui sera sérieusement considérée comme un complément aux médicaments disponibles pour la prise en charge des patients atteints de COVID-19.

Mots clés: ivermectine; pharmacocinétique; pharmacodynamique; antiviral à large spectre; COVID-19

Introduction:

Ivermectin, an anti-parasitic agent has been used for several years to treat many infectious diseases in mammals due its broad-spectrum antimicrobial activity and high therapeutic efficacy (1). It has a wide safety margin with low adverse effects when it is used orally. Ivermectin is a dihydro derivative of avermectin that was discovered in the late 1970s and this drug was first approved for use in veterinary medicine in 1981 (2). Its potential use in humans was confirmed in 1987 for the treatment of onchocerciasis. Subsequently, William Cecil Campbell, an Irish American scientist and Satoshi Ōmura, a Japanese biochemist both of whom discovered and developed this medication were honored with the 2015 Nobel Prize in Physiology or Medicine (3).

Ivermectin is currently used as an approved drug in several countries for the treatment of filariasis, strongyloidiasis, scabies and onchocerciasis. As a result of mass drug distribution and administration programmes, an estimated 3.7 billion doses of ivermectin have been distributed globally over the past thirty years (4). Studies have suggested the biochemical property of ivermectin as a broad-spectrum drug with high lipid solubility. This characteristic confers on it multipurpose effects on several parasites and viruses through a variety of mechanisms. Apart from the anti-parasitic and antiviral effects, ivermectin also causes immunomodulation in the host cells. Other studies have corroborated the effect of ivermectin on slowing down the proliferation of cancer cells, including the regulation of glucose and cholesterol in animals (5).

It has also been suggested in studies that the inhibitory action of ivermectin on the integrase protein of HIV (6), and non-structural protein 5 in Dengue virus (7) can inhibit their replication. Also, ivermectin has demonstrated inhibition against several RNA viruses such as West Nile virus (8), Venezuelan equine encephalitis virus (9), and influenza virus (10). The antiviral effect of ivermectin has also been demonstrated, both *in vitro* and *in vivo*, against pseudorabies virus (PRV), which is a DNA virus

(11). The nuclear inhibitory activity of ivermectin appears to be responsible for its broad-spectrum activity against these viruses (12).

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, which is officially known as coronavirus disease - 2019 (COVID-19) by the World Health Organization (WHO) was first reported in early December 2019 in Wuhan, China and has subsequently spread globally. Since the outbreak of the virus infection, there has not been any 'gold standard' antiviral drug available for its treatment. However, there are many ongoing clinical trials exploring the potential antiviral activities of a number of drugs. Several of these drugs, which were initially used to eliminate other pathogens, now appear to have shown some inhibitory activities against COVID-19 either by direct action on the viral agent or by modulating the host immune system. Ivermectin is one of those drugs with potential therapeutic activity in COVID-19 as it has demonstrated potential antiviral activity by its action against the nuclear transport of viral proteins (13). The objective of the present study is to review the antiviral activities of ivermectin with the aim of finding an additional drug that may be useful in reducing the morbidity and mortality from the dreaded COVID-19.

Materials and method:

Online literature search was conducted on Google, Google Scholar, JSTOR, and EBSCO for relevant publications on ivermectin use in COVID-19 and for other viral infections. Publications were evaluated based on credibility of the sources, innovative contribution, key concepts and theories, and problem-solving approach, using the Preferred Reporting Items for Systematic Review and Meta-Analysis guide as shown in Fig 1.

Recurring themes were identified, debates, conflicts, contradictions and identified gaps in knowledge were noted, as there were gaps in combinational use of ivermectin with other drugs, as well as about its efficacy. The outline of structure of the review was discussed amongst all authors.

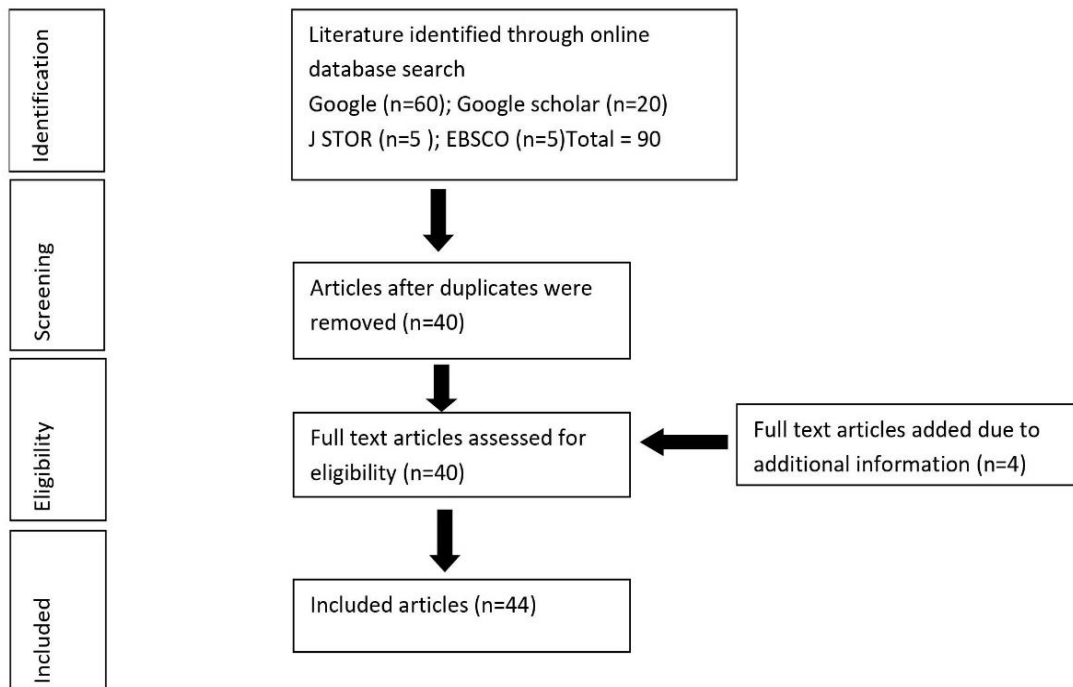


Fig 1: Process of selection of publications (PRISMA guide) used for the review

Chemistry of ivermectin

Ivermectin is a semi-synthetic broad-spectrum anti-helminthic, anti-viral and anti-cancer agent. It is an avermectin, which is a group of pentacyclic sixteen-membered lactone (macrocyclic lactone disaccharide) (Fig 2), derived from the soil bacterium *Streptomyces avermitilis*. The simple derivative of the mixture of the natural compounds, avermectin B1a and B1b, are the most common avermectin.

The peculiar chemistry of ivermectin may make possible for two ivermectin molecules to create a complex to be considered as ionophore. According to this hypothesis (14), this ionophore for cations such as zinc for example, may then affect the hydro-electrolyte balance in the cell to cause early virophagy and even act to inhibit RNA-dependent-RNA polymerase (RdRp), similar to remdesivir (15).

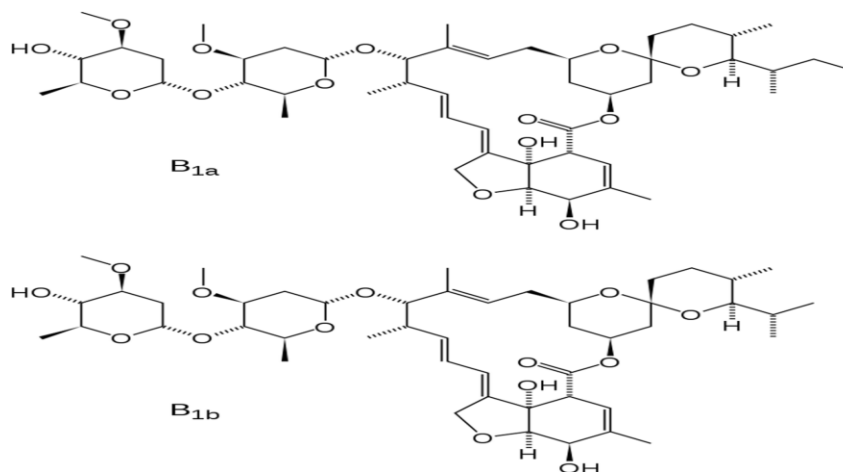


Fig 2: Structural formulas of ivermectin compounds

Pharmacokinetics of ivermectin

Similar to azithromycin, ivermectin is highly lipophilic and usually administered orally and rapidly absorbed. It reaches peak plasma concentration (T_{max}) in 4 hours, and is 93% protein bound with a half-life of 16 hours and terminal half-life of 81-91 hours. Its volume of distribution is 3.5 L/kg and undergoes entero-hepatic circulation. After a single oral dose of 150 $\mu\text{g}/\text{kg}$, the C_{max} is 40 ng/ml and T_{max} is 4 hours. These remain unchanged after a dose of 200 $\mu\text{g}/\text{kg}$ (16). Plasma concentration achieved with a maximum tested dose of 2 mg/kg was 250 ng/ml. The researchers have speculated that the projected therapeutic level of 2,500 ng/ml for *in vivo* efficacy in COVID-19 may be impractical. Other researchers concluded that the likelihood of clinical treatment using the approved dose of ivermectin is low, since doses 10x higher than the approved dose cannot meet the concentration that will result in 50% inhibition (IC_{50}) (17).

Ivermectin is metabolized by the liver microsomes P450 and converted to mostly hydroxylated and demethylated metabolites which are 90% excreted in faeces, with 1% in urine (18). There is risk of absorption through the blood-brain-barrier (BBB) when taken with other CYP3A4 inhibitors such as statins, HIV-1 protease inhibitors, calcium channel blockers (CCBs), lidocaine, benzodiazepines, and glucocorticoids like dexamethasone which also inhibit p-glycoprotein. This is pertinent because ivermectin is a substrate and an inhibitor of p-glycoprotein (19), thus the neurotoxicity of ivermectin may be marked at high dose.

Doxycycline enhances ivermectin-induced suppression of microfilaridemia while ivermectin antagonizes vitamin K to increase prothrombin time. Ivermectin does not readily cross the BBB due to the efflux transporter, p-glycoprotein (20). Large doses may also not be tolerable for humans because of the inhibition of the nuclear transporter, importin or karyopherin α/β I (21,22). Importins are regulatory proteins vital for gametogenesis, embryogenesis and prevention of cancer.

The Food and Drug Administration (FDA) and Pan-American Health Organisation (PAHO) in 2020 have not approved ivermectin for use in COVID-19. Ivermectin is contraindicated in children under 5 years of age or in children with weight less than 15 kilograms (33 pounds) and in individuals with liver or kidney disease (23).

Pharmacodynamics of ivermectin

Ivermectin is an agonist for the gamma aminobutyric acid (GABA)-gated chloride channels, stimulating release of GABA from the pre-synaptic end of the GABAergic nerves. It thus enhances the post-synaptic GABA binding and amplifies GABA function in the CNS. This may explain its side-effects of dizziness, ataxia and fatigue. The killing of parasites by ivermectin is due to its agonist action at the glutamate-gated chloride channel (24).

Ivermectin has shown *in vivo* effects against RNA viruses such as the West Nile virus and Newcastle disease (NCD) virus at a concentration of 100 $\mu\text{g}/\text{ml}$ through inhibition of importin Imp α/β I. This may also explain its action against SARS-COV2 (25). It has also been reported to inhibit *in vivo* DNA viruses such as pseudorabies virus (PRV) and parvovirus by preventing nuclear import of UL42, an accessory subunit of the DNA polymerase.

Ivermectin induction of autophagy may explain its action as an anti-viral agent. Through the induction of autophagy as calorie restriction does, it may directly eliminate intruding viruses (virophagy), enhance immune cell competence, suppress acute inflammation and hyper-inflammation (26,27). Importantly, ivermectin activates AMP-activated protein kinase (AMPK) and upregulates Farnesoid X receptor (FXR) to decrease expression of lipogenesis-related genes (28,29) and this mechanism could help attenuate SARS-COV-2 virus entry. Ivermectin is active against SARS-COV that is homologous to SARS-COV2, by inhibiting host importin protein, Imp α/β I heterodimer, which is the major transporter in nucleocytoplasmic shuttling of SARS-COV nucleocapsid protein. The new hypothetical mechanism to explain anti-COVID-19 effect of ivermectin is that it displays ionophore activity to cause early viral lysis (27).

Clinical uses of ivermectin

Ivermectin is currently used as an approved drug in several countries for the treatment of filariasis, strongyloidiasis, scabies and onchocerciasis (4). It also exhibits action against West Nile virus (8), Venezuelan equine encephalitis virus (9), influenza virus (10) and pseudorabies virus (11,13). Although the drug has not yet gained FDA and PAHO approval for use in COVID-19, clinical trial of ivermectin alone (30) or the combination of zinc, doxy-

cycline and ivermectin in COVID-19, is however recruiting participants (NCT04482686). A previous study (31) showed that a single dose of ivermectin was enough to limit SARS-CoV-2 replication within 24-48 hours; this action was possibly due to the inhibition of the IMP α / β 1-mediated nuclear import of viral proteins. The same study also demonstrated that the levels of viral RNA released from the infected cells and cell-associated viral RNA were reduced profoundly by more than 90% and 99% respectively at 24 hours post SARS-CoV-2 infection. In addition to these, the treatment of SARS-CoV-2 infected cells with ivermectin for 48hrs was demonstrated to lead to approximately 5000-fold reduction of viral RNA in comparison with the control group. However, the study reported that no further reduction in the viral RNA was demonstrated at 72 hours (13). Furthermore, it has been proposed that no toxicity of ivermectin was seen in both the test and control groups at any point in time (31).

Benefits of ivermectin in management of viral infections

Ivermectin has antimicrobial, antiviral and anti-cancer properties, which gives it the ability to treat a range of diseases. The drug was approved by the FDA as a broad spectrum anti-parasitic agent (32). Ivermectin has been shown to have antiviral activity against a broad range of viruses both RNA and DNA viruses (33). Examples of RNA viruses include dengue virus, Zika virus, Yellow fever virus, West Nile virus, Hendra viruses, Newcastle virus and SARS-coronaviruses. Examples of DNA viruses include Equine herpes virus type 1, BK polyoma virus, and bovine herpes virus (BHV) (32).

Antiviral effects of ivermectin on RNA viruses

Dengue virus

Dengue virus (DENV) is a positive (+) sense, single stranded RNA virus of genus flavivirus and family Flaviviridae. In an *in vitro* study carried out on human cervical adenocarcinoma cells (HeLa) which was infected with Dengue virus, ivermectin at high concentration (25-50 μ M) had an inhibitory effect on proliferation of the virus. This action was achieved by inhibition of the transfer of viral proteins between host cell cytoplasm and its nucleus, which is dependent on IMP α / β 1 (importin). It was observed that ivermectin inhibited the nuclear aggregation of NS5 protein of Dengue virus, the largest (102 kDa) and the most conserved protein, with approximately 70%

sequence identity among the four Dengue serotypes expressed during infection by Dengue virus (34).

In a study conducted on Yellow Fever, West Nile and Dengue viruses, ivermectin exerted its inhibitory effect by inhibiting NS3 helicase (which mediate RNA binding and unwinding mechanism). It however did not have any effect on ATPase activity of the helicase domains. Ivermectin showed stronger inhibitory effect on Yellow fever virus, and to a lesser extent, inhibited proliferation of West Nile virus and Dengue virus. It was confirmed that ivermectin exerts its effect against dsRNA unwinding activity by acting on the flavivirus helicase enzyme. It however did not affect the ATPase activity because ATP is a key nucleotide in host cell metabolism (34).

Zika virus

Zika virus is a single stranded (ss) RNA virus of genus flavivirus and family Flaviviridae. It was studied by Barrow et al., (35), who performed the *in vitro* study on Zika-infected Huh-7 cells (ZIKMEX_1_7) and confirmed the antiviral effect of ivermectin on the virus.

SARS-CoV-2

SARS-COV-2 is a novel virus that was discovered in December 2019. In an *in vitro* study conducted on the virus, vero/hSLAM cells infected with the SARS-CoV-2 were exposed to 5 μ M ivermectin in 48 hours and a 500-fold reduction in viral RNA compared with the control was found (36). The study showed that treatment with ivermectin effectively kills almost all viral particles within 48 hours. It was acknowledged that the drug may have antiviral effects by inhibiting the importin (IMP) α / β receptor, which is responsible for transmitting viral proteins into host cell nucleus (36).

HIV-1

HIV-1 is a single stranded RNA virus belonging to the genus Lentivirus of the family Retroviridae. In an *in vitro* study on the effect of ivermectin as an inhibitor of HIV-1 nuclear protein transfer, it was observed to reduce the nuclear localization signal (NLS)-containing protein binding by IMP α / β and inhibited this interaction at low concentrations. Ivermectin significantly ($p=0.003$) reduced nuclear accumulation of the green fluorescent protein (GFP-IN) compared to untreated control group, and also significantly reduced ($p<0.001$) nuclear accumulation of GFP-tagged Op-T-NLS fusion protein. Ivermectin however failed to control nuclear accumulation of telomere repeat factor-1 (GFP-TRF) as IMP β 1 is the only way to transfer it to the cell nucleus. The study concluded

that ivermectin is a nuclear transport inhibitor, via IMP β 1 alone, and also, it completely inhibited nuclear import of the active integrase protein of HIV-1 as a critical component of the pre-integration complex (37). Kylie et al., (34) study on HIV-1 infected human cervical adenocarcinoma cells (HeLa) showed that ivermectin at high concentrations (25-50x) had inhibitory effect on proliferation of HIV-1, which was achieved by inhibiting transfer of viral proteins between the host cell cytoplasm and its nucleus, dependent on IMP α / β 1. It was also shown that ivermectin inhibited nuclear aggregation of HIV-1 integrase (34).

Newcastle disease virus

Newcastle disease (NCD) virus is a negative sense, single stranded virus of the family Paramyxoviridae. Azeem et al., (38) studied the cytotoxicity of ivermectin and its potential antiviral effect on the NCD virus by using chick primary fibroblast cell line and 9-day old chick embryo. Ivermectin was tested at concentrations of 6.25, 12.5, 25, 50, 100, 200 μ g/ml. The results showed that the drug at 100 μ g/ml or above had cytotoxic effect but was safe at 50 μ g/ml or less. At this dose, drug cytotoxicity was not observed and a moderate to poor antiviral activity was noted.

Antiviral effects of ivermectin on DNA viruses

Equine herpes virus type 1 (EHV-1)

EHV-1 is a double stranded DNA virus. In primary murine neurons infected with 2 different strains of EHV-1, Jan-E and Rac-H, different concentrations of ivermectin had no effect on strain Rac-H proliferation but reduced the proliferation of strain Jan E, suggesting that different strains of EHV-1 use different receptors to enter the nucleus (39).

Pseudorabies virus (PRV)

PRV is an enveloped double stranded DNA based swine virus belonging to α -herpesviridae subfamily, which causes lifelong infection in pigs. Its DNA polymerase enzyme is made up of two subunits UL30 and UL42 (40,41). UL42 subunit has IMP- α / β -mediated bipartite nuclear localization signals (NLS) which transfer both units into the nucleus (41). Examination of infected baby hamster kidney (BHK-21) cells showed that ivermectin did not produce cytotoxic effects at a concentration <3 μ M. Increasing the concentration to 5 μ M showed drug cytotoxic effect and sharp reduction in cell activity.

Ivermectin inhibited the entry of DNA

polymerase accessory subunit UL42 into the nucleus, so that with increasing drug concentration, less UL42 was observed in the nucleus by Western blot analysis method. Ivermectin inhibited transfer of UL42 to the nucleus through the NLS but did not reduce UL42 expression in the cytoplasm (41). In the virus-infected mice model (*in vivo* studies), ivermectin significantly reduced viral loads in the brain and kidney of all animals; the reduction was more significant in the kidneys, the main organ involved in ivermectin metabolism. In addition to the declining virus titers in the organs of the animal, their clinical scores and mortality decreased as the drug concentration increased. It was therefore concluded that ivermectin could be used as a potential antiviral drug against PRV infection (42).

BK polyomavirus (BkPyV)

Bennet et al., (43) studied the effect of ivermectin on BkPyV, a non-enveloped small double stranded (ds) DNA virus belonging to the family Polyomaviridae, in infected epithelial cells of the proximal convoluted tubule (PCT) of the kidney. This was a qualitative study that used reverse transcription-PCR after treating infected cells with 10 μ M ivermectin. The study showed a reduction in the level of early protein large T antigen mRNA, indicating a reduction of viral gene expression due to the inhibition of nucleus entry. This inhibitory effect of ivermectin indicates that polyomavirus has access to the nucleus through active pore complex transfer (43).

Bovine herpes virus- 1 (BOHV-1)

In one study (44), Madin-darby bovine kidney cells were infected with BOHV-1, a large enveloped double stranded DNA virus from family herpesviridae. Ivermectin decreased UL42 nuclear transmission by inhibiting IMP α / β dependent nuclear transfer and reduced virus replication in a dose dependent manner. This indicated that UL42 was dependent on IMP α / β for nuclear transfer. Ivermectin at 25 μ M reduced the virus titer by 4 logs and inhibited virion production by 44%, but had no effect on cell viability at the studied doses. Ivermectin also had no effect on binding and entry of virus into host cell (44).

Discussion:

Ivermectin has been shown to have antiviral activity against a broad range of RNA and DNA viruses (32,33). Examples of such RNA viruses include Dengue virus, Zika virus, Yellow fever virus, West Nile virus, Hendra

viruses, Newcastle virus and SARS viruses, and examples of DNA viruses include Equine herpes type 1, BK polyoma virus and bovine herpes virus (32,33). In *in vitro* studies (34), ivermectin exerted inhibitory effects by inhibiting NS3 helicase (which mediate RNA binding and unwinding mechanism). The drug has also demonstrated *in vitro* activity against other viruses such as HIV-1 (37), equine herpes virus type 1 (39), pseudorabies virus (40,41), BK polyomavirus (43) and bovine herpes virus -1 (44).

Ivermectin is currently used as an approved drug in several countries for the treatment of filariasis, strongyloidiasis, scabies and onchocerciasis. An estimated 3.7 billion doses of ivermectin have been distributed globally over the past thirty years (4). It is an avermectin, which is a group of pentacyclic 16-membered lactone (macrocyclic lactone disaccharide). It is believed that the peculiar chemistry of ivermectin makes it possible for two ivermectin molecules to create a complex to be considered as ionophore (14).

An ionophore for cations such as zinc for example may then affect the hydro-electrolyte balance in the cell to cause early viroplasm and even act to inhibit RNA-dependent-RNA polymerase (RdRp) as done by remdesivir (15). It could therefore be expected that when administered with zinc, ivermectin should be able to interfere with the replication process of SARS-CoV-2. The new hypothetical mechanism to explain anti-COVID-19 effect of ivermectin is that it displays ionophore activity to cause early viral lysis (27). Clinical trials of ivermectin alone (30) or the combination of zinc, doxycycline and ivermectin on COVID-19 management are currently recruiting participants (27). It is therefore recommended that more *in vitro* and *in vivo* studies be carried out to determine the clinical efficacy of ivermectin in SARS-COV-2 infections, with the aim of reducing morbidity and mortality from the ongoing COVID-19 pandemic.

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