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(54) Title: NICLOSAMIDE FOR THE TREATMENT OF VIRAL DISEASES

(57) Abstract: The present invention provides pharmaceutical composition comprising niclosamide for use in the therapeutic or prophylactic treatment of viral diseases or conditions. Viral diseases treated or prevented by the compositions of the present invention can be Ebola virus disease, MERS (MERS-CoV), SARS (SARS-CoV), SARS-CoV-2 (COVID-19) virus disease, Rabies, viral diseases caused by Orthomyxoviridae family virus including influenza A, influenza B, influenza C, influenza D virus disease, hepatitis A, Rhinovirus, Marburg virus disease, hepatitis C, Yellow fever virus disease, Dengue fever virus disease, West Nile virus disease or ZIKA virus disease.

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NICLOSAMIDE FOR THE TREATMENT OF VIRAL DISEASES

TECHNICAL FIELD

- 5 The present invention provides a pharmaceutical composition comprising niclosamide for use in the therapeutic or prophylactic treatment of viral diseases.

BACKGROUND ART

Niclosamide, or 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide, is an efficacious, minimally toxic and FDA-approved anti-helminthic drug that has been used in patients for
10 decades. The anti-parasitic activity of niclosamide was originally reported to be mediated by inhibition of mitochondria oxidative phosphorylation and anaerobic ATP production [Weinbach, 1969].

There has been increased interest in niclosamide's action against key pathological pathways due to the fact that niclosamide not only inhibits the Wnt/ β -catenin, mTORC1, STAT3, NF- κ B and
15 Notch signaling pathways, but also targets mitochondria in cancer cells to induce cell cycle arrest, growth inhibition and apoptosis (Li et al., Cancer lett (2014)10;349(1):8-14). Thus, the use of niclosamide is suitable for almost all types of cancer. Niclosamide has also shown great potential for the treatment of viral diseases and was found to be effective against various viral infections such as SARS-CoV, MERS-CoV, ZIKV, HCV, and human adenovirus, indicating
20 its potential as an antiviral agent and also holds great potential for the treatment of COVID-19 (Xu et al, 2020, "Broad Spectrum Antiviral Agent Niclosamide and its therapeutic Potential").

Luo et al, published an article in Journal of Medical Virology on 22 January 2020 about global health concerns stirred by emerging viral infections saying; "Emerging viral infections continue to pose a major threat to global public health. In 1997, a highly pathogenic avian influenza A
25 (H5N1) virus was found to directly spread from poultry to humans unlike previously reported, other avian influenza A virus subtypes (H7N9, H9N2, and H7N3) were also associated with human disease, raising an alarm that all subtypes of influenza A virus circulating in domestic and wild birds and livestock can potentially spill over to humans, resulting in pandemics.

The outbreak of severe acute respiratory syndrome (SARS) happened during 2002 to 2003 in
30 China was caused by a novel coronavirus (CoV) designated as SARS-CoV, spreading to 37

countries and resulting in more than 8000 infections and 774 deaths (9.6% mortality rate). 6 more recent years have witnessed the emergence of several other important viral diseases, including a pandemic influenza caused by a swine H1N1 influenza A virus in 2009, the Middle East respiratory syndrome (MERS) caused by a new deadly (>30% mortality) MERS-CoV in 5 2012, the Ebola outbreak in West Africa during 2014 to 2016, and the microcephaly crisis associated with Zika virus infection in 2015.”

Coronaviruses (CoVs) are enveloped and positive-sense single-stranded RNA viruses belonging to the family Coronaviridae within the order Nidovirales. Human coronavirus infections are typically mild and rarely associated with severe diseases. However, the epidemics 10 of Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) caused alarming morbidity and mortality. While coronaviruses are often zoonotic, person-to-person transmission has been confirmed for SARS-CoV-2, similar to MERS-CoV and SARS-CoV.

Since new human coronavirus disease has emerged at the end of December 2019, novel 15 treatment options are still needed globally. To date, any highly effective treatment method has not been found yet. During this overwhelming outbreak, scientists are still seeking a way to stop this viral infection and a remarkable number of scientists are focusing on repositioning/repurposing approach which defined as relocating an existing treatment against a new condition or disease other than its’ indication.

20 Thus, it is of grave importance to develop new therapies for the treatment of viral diseases, especially viral RNA infections including SARS-CoV, MERS-CoV, ZIKV, HCV and most importantly 2019-nCoV, which has become a global pandemic.

In recent literature, there are numerous repurposing studies of niclosamide targeting viral strains. Furthermore, there is a newly presented drug discovery study based on artificial 25 intelligence (AI)/machine learning (ML) approach pointing potential anti-SARS CoV-2 activity of Niclosamide (Jurgeit et al., PLoS Pathog. 2012 Oct; 8(10): e1002976)

The usual oral dose of niclosamide is 2000 mg as a single dose daily for 7 days, followed by 1000 mg for 6 days; including pediatric patients 6 years and older. But the use of drug is limited by side effects. Niclosamide is absorbed from the gastrointestinal tract and tablets are given on 30 an empty stomach in the morning in order to enhance its dissolution and absorption. But, this dosage regime generally causes gastrointestinal side effects.

In a study evaluating antiviral activity of niclosamide, activity against SARS-CoV strains of the molecule was demonstrated in vitro (Xu et al., ACS Infect. Dis 2020, 6, 909-915). In another antiviral activity study, the antiviral potential of niclosamide was shown against various virus strains including influenza, in vitro (Antimicrobial Agents and Chemotherapy 48(7):2693-6).

- 5 In regard of these evidences including in vitro results ($IC_{50} = 3.12 \mu M$) against SARS CoV strains presented and plasma concentration estimation of 400 mg oral dose ($3.6 \mu M$) is indicating that 400 mg oral dose of niclosamide is an adequate dose for effective concentration.

There are also ongoing clinical studies for the use of niclosamide in the treatment of Covid-19. NCT04399356 numbered clinical trial is now on phase 2 and evaluate niclosamide for the
10 treatment of Covid-19. Participants to trial receive 2000 mg of niclosamide in a day. The other clinical trial evaluates niclosamide with the combination of diltiazem (NCT04372082). Despite the combination, the daily dosage of niclosamide is still 2000 mg.

However, the use of niclosamide has a major disadvantage; low solubility, low bioavailability and poor pharmacokinetic profile, which results in limited efficacy as a therapeutic for human
15 use in the viral disease indication. In press release of Institut Pasteur and Daewoong Holding declare that they are developing niclosamide for the COVID-19 treatment; despite its excellent antiviral effect on COVID-19, niclosamide had a problem of maintaining blood drug concentrations in the human body when taken orally, making it difficult to be applied as an actual treatment for COVID-19.

20 In addition, the clinical trial of niclosamide for prostate cancer (PLos One, 2018 August 15;13(8):e0202709) demonstrated decreased cancer cell proliferation across multiple cell lines. Due to the promising in-vitro results, one would expect, at least a moderate level of success. But in the words of the principal investigator of the study: "Because niclosamide plasma concentrations in the maximal tolerated dosing cohort (i.e., 500 mg TID) were below those
25 expected to exert an anti-tumor effect, the study was closed for futility." Which creates another problem to be solved; niclosamide can cause side effects when administered for a long period of time. Thus, the dose cannot surpass 2000mg a day, or even 1500mg a day as side effects start occurring even at this daily dose level.

It is clear from the prior art that niclosamide shows serious side effects depending upon the
30 daily doses and in addition its effectiveness depends on its dissolution and solubility profile. Thus, there is still a need in the art for a pharmaceutical composition which is effective in viral

diseases, and also which is safe to use without side effects; in addition which is also stable, easy to manufacture and economically more viable, so it can be mass produced with ease in situations like the 2019-nCoV pandemic.

DETAILED DESCRIPTION OF THE INVENTION

- 5 The present invention provides pharmaceutical composition comprising niclosamide for use in the therapeutic or prophylactic treatment of viral disease.

The viral disease is caused preferably by RNA viruses. Most viruses are classified into broad categories based on the types of nucleic acids formed during replication and the pathway by which mRNA is produced. In general, viruses have either RNA or DNA as their genetic
10 material, wherein the nucleic acid can be single- or double-stranded.

Important virus families of the DNA type include adenoviridae, herpesviridae, poxviridae, papovaviridae, densovirinae, and parvovirinae. Virus families typically classified of the RNA type include birnaviridae, reoviridae, astoviridae, arterivirus, caliciviridae, coronaviridae, flaviviridae, picornaviridae, togaviridae, polioviruses, bornaviridae, filoviridae,
15 paramyxovirinae, pneumovirinae, rhabdoviridae, bunyaviridae, and orthomyxoviridae.

According to the present invention, the pharmaceutical composition is used for the treatment of viral diseases caused by single stranded (ss) RNA viruses.

Conditions or diseases that can be treated with the composition of the present invention include but are not limited to the Ebola virus disease, SARS, MERS, COVID-19 virus disease, Rabies,
20 viral diseases caused by Orthomyxoviridae family virus including influenza A, influenza B, influenza C, influenza D virus disease, hepatitis A, Rhinovirus, Marburg virus disease, hepatitis C, Yellow fever virus disease, Dengue fever virus disease, West Nile virus disease and ZIKA virus disease; preferably MERS (MERS-CoV), SARS (SARS-CoV), SARS-CoV-2 (COVID-19), influenza A, influenza B and influenza C and most preferably SARS-CoV-2 (COVID-19),
25 influenza A, influenza B and influenza C.

According to the present invention, the pharmaceutical composition may comprise niclosamide in an amount of between 40 to 400 mg, preferably 150 to 300 mg and more preferably 160 to 240 mg.

In one preferred embodiment of the present invention, niclosamide is administered in a maximum daily dosage of 1200 mg, preferably 900 mg, more preferably 750 mg and most preferably 600 mg

5 In the prior art, niclosamide is administered in daily doses of 2000 mg. It has been surprisingly found that when niclosamide is administered in the amount and with the regime according to the present invention for use in the treatment of RNA virus diseases or conditions, the safe, non-toxic and efficient treatment is provided. The amount of niclosamide has been reduced in the composition. It would be expected that the amount of niclosamide would not be enough and the treatment would not be achieved, but the inventors found that therapeutic doses in the
10 present composition are strong enough to deliver effective treatment and that the total daily dose of niclosamide is lower than prior art's daily dose which is 2000 mg. The effective treatment is achieved with the total daily dose of niclosamide administered preferably less than 900 mg due to the superior solubility and significant bioavailability that can be attained.

It has been also found that side effects do not occur at the daily dose level according to the
15 present invention. The bioavailability of niclosamide is increased compared to the bioavailability of the same amount of niclosamide formulated according to the prior art. The low solubility and dissolution profile lead to low bioavailability of niclosamide. Therefore, in the prior art, very high oral doses and repeated dosing have to be used to obtain effective blood concentrations, which creates toxicity and other side effects such as Nausea, Anorexia,
20 Vomiting, Diarrhea, Weight loss, Lipase elevation, Colitis and Abdominal pain. But side effects and toxicity problems are overcome using niclosamide in the pharmaceutical formulation of the present invention. Niclosamide can be effectively used to treat viral diseases at doses according to the present invention with low toxicity. Toxicity test results are given below.

LD50 as an index of acute toxicity is less than 2500 mg/kg in male and female mice according
25 to the present invention.

In one preferred embodiment of the present invention, niclosamide can be administered 2 to 4 times daily. Thus, the treatment regimen for niclosamide will be twice or thrice a day with a total daily dose between 80 and 900 and preferably 200 and 600 mg.

As it would be known by the person skilled in the art; the dose to be administered can also be
30 calculated based on the weight of the patient. In that case, the dose of niclosamide is between 2,46 mg/kg and 3,69 mg/kg.

Especially, contrary to the planned human clinical studies for the treatment of Covid-19, the preferred embodiment of the present invention entails that niclosamide is administered 3 times a day to provide a constant inhibitory concentration with a decrease in dose dependent toxicities and side effects.

5

The pharmaceutical composition according to the present invention may be formulated as an oral unit dosage form. The oral unit dosage forms of pharmaceutical compositions can be administered one or more times daily, preferably 2 to 4 times daily. 2 to 4 times daily administration and most preferably 3 times daily administration of the composition is essential to the success of the treatment, since niclosamide is rapidly eliminated by the kidneys and the half-life of niclosamide is 6.0 ± 0.8 hours and the 3 times a day application creates the necessary inhibitory concentration throughout the treatment period.

10

According to the present invention, the pharmaceutical composition further comprises at least one emulsifier. Within the scope of the present invention, it has been found that the use of an emulsifying increases the solubility of niclosamide and plasma concentration of niclosamide; thus, the bioavailability of niclosamide.

15

The emulsifier of the present invention has an HLB value between 10 and 25, preferably between 10 and 21.

As used herein, HLB means hydrophilic-lipophilic balance (HLB), i.e. the balance of the size and strength of the hydrophilic (water-loving or polar) and the lipophilic (oil-loving or non-polar) groups of the emulsifier. In the HLB system, each emulsifier is assigned a numerical value which is called its HLB. The HLB of emulsifiers is shown in all current ICI emulsifier literature and similar values may be calculated or estimated by various means for any emulsifier. All emulsifiers consist of a molecule that combines both hydrophilic and lipophilic groups. An emulsifier that is lipophilic in character is assigned a low HLB number (below 9.0), and one that is hydrophilic is assigned a high HLB number (above 10.0). Those in the range of 9-10 are intermediate.

20

25

The emulsifier is preferably selected from the group consisting of polyoxethylene derivatives, sorbitan esters, polyethylene glycol derivatives and a combination thereof. Polyoxethylene can be polyoxyglycerides such as stearyl polyoxyl-32 glycerides, lauroyl polyoxyl-32 glycerides or polyoxy-ethylene sucrose diester dimyristate, Polyoxy-ethylene sucrose diester dinnyristate,

30

polyoxy-ethylene sucrose diester dipalmitate, polyoxy-ethylene sucrose diester dioleate; sorbitan esters can be polysorbate 80, polysorbate 60, polysorbate 20; polyethylene glycol derivatives can be PEG-8 laurate, PEG 400 monoluarate, PEG 10 isooctylphenyl ether, PEG 40 stearate, PEG 50 stearate, PEG 40 isooctylphenyl ether, PEG-25 Castor Oil, PEG-30 Castor Oil, PEG-40 Castor Oil, PEG-25 Hydrogenated Castor Oil, PEG-60 Hydrogenated Castor Oil, Hexylene Glycol with PEG-25 Hydrogenated Castor Oil (and) PEG-40 Hydrogenated Castor Oil.

The emulsifier is preferably polyoxylglycerides or polysorbates or polyethylene glycol derivative. Thus, the emulsifier used in the present composition is preferably selected from; polysorbate 80, polysorbate 60, polysorbate 20, stearyl polyoxyl-32 glyceride (Acconon C-50/ Gelucire 50/13) or lauroyl polyoxyl-32 glyceride (Acconon C-44/ Gelucire 44/14), PEG-8 laurate, PEG 400 monoluarate, PEG 10 isooctylphenyl ether, PEG 40 stearate, PEG 50 stearate, PEG 40 isooctylphenyl ether, PEG-25 Castor Oil, PEG-30 Castor Oil, PEG-40 Castor Oil, PEG-25 Hydrogenated Castor Oil, PEG-6Hydrogenated Castor Oil, Hexylene Glycol with PEG-25 Hydrogenated Castor Oil (and) PEG-40 Hydrogenated Castor Oil.

Although substantially high levels of solubility can be achieved with the composition of the present invention comprising an emulsifier such as a sorbitan ester (polysorbate 80),

Thus, the pharmaceutical composition of the present invention further comprises dextrin compound as well as an emulsifier.

It has also been surprisingly discovered that polyoxyethylene derivatives and preferably sorbitan esters can have an even stronger effect on the solubility of niclosamide, when combined with a dextrin. The amphiphilic nature of sorbitan esters, combined with their high HLB value, results in this synergistic composition with a highly soluble and bioavailable niclosamide composition.

Dextrin compounds are a group of low-molecular-weight carbohydrates produced by the hydrolysis of starch or glycogen.

One preferred type of dextrin of the present invention is maltodextrin and the others are cyclodextrins.

Maltodextrin is a short-chain starch sugar used as a food additive in prior art. It is produced also by enzymatic hydrolysis from gelled starch and is usually found as a creamy-white hygroscopic

spray-dried powder. Maltodextrin is easily digestible, being absorbed as rapidly as glucose, and might either be moderately sweet or have hardly any flavor at all.

The cyclical dextrans are known as cyclodextrins. They are formed by enzymatic degradation of starch by certain bacteria, for example, *Paenibacillus macerans* (*Bacillus macerans*).

5 Cyclodextrins have toroidal structures formed by 6-8 glucose residues.

Accordingly, the preferred dextrin compounds of the present invention are selected from beta cyclodextrin and derivatives including but not limited to: β -cyclodextrin, 2-hydroxypropyl- β -cyclodextrin, sulfobutylether β -cyclodextrin sodium salt, randomly methylated β -cyclodextrin,
10 branched β -cyclodextrin and maltodextrin.

Although γ -cyclodextrin compounds can also be employed, the preferred embodiment of the invention entails the use of beta cyclodextrins including; beta cyclodextrin (BCD), DM- β -cyclodextrin, RM- β -cyclodextrin and hydroxypropyl β -cyclodextrin (HPBCD), which have enhanced the solubility of niclosamide even with the least expensive cyclodextrin compound
15 β -cyclodextrin. In addition, formulations in the prior art have focused on extremely high ratios of cyclodextrin compounds such as HPBCD, contrary to the findings of the present invention that provides significantly higher levels of solubility with lower amounts of cyclodextrin compared to state of the art, when combined with emulsifiers that have a high HLB value.

It has also been surprisingly discovered that polyoxyethylene derivatives and preferably
20 sorbitan esters can have an even stronger effect on the solubility of niclosamide, when combined with a dextrin. The amphiphilic nature of sorbitan esters, combined with their high HLB value, coupled with the hydrophilic nature of dextrans, results in this synergistic composition with a highly soluble and bioavailable niclosamide composition.

According to the present invention, 1% to 6% of niclosamide is released within 30 minutes or
25 preferably within 60 minutes when subjected to an in vitro dissolution test at 100 rpm in 900 mL of pH 6.8 phosphate buffer and at 37.0°C. $\pm$ 0.5°C.

In another embodiment of the present invention, the pharmaceutical composition may be used in combination with other active ingredients used in the treatment of viral disease, especially Covid-19. When combined with other drugs, the safety profile of niclosamide is very
30 advantageous.

Niclosamide is eliminated by the kidneys. Considering that all of the antiviral drugs used in the treatment of Covid-19 such as oseltamivir, azithromycin and hydroxychloroquine sulphate are metabolized in the liver, elimination of niclosamide by the kidney is important for the safety of the treatment protocol. In addition, niclosamide may reduce the nephrological damage of other
5 antiviral drugs. It has been demonstrated by animal and cell experiments that, instead of creating nephrotoxicity in kidneys, niclosamide reaching the kidney has an anti-inflammatory effect (Mol Med Rep. 2017 Aug; 16(2): 1810-1816) and reduces fibrous tissue formation (Kidney Int. 2017 Sep; 92(3):612-624).

Accordingly, the pharmaceutical composition may be combined with at least one antiviral or at
10 least one antibiotic compound.

Accordingly, the composition of the present invention may be administered before/after/during treatment with favipiravir, oseltamivir, hydroxychloroquine sulphate, chloroquine phosphate, lopinavir/ritonavir, remdesivir, interferon alpha and interferon beta, azithromycin, budesonide, paracetamol and dexamethasone.

15 For the avoidance of doubt, the term “before/after/during” means that the niclosamide composition of the present invention is administered during another interventional treatment or within 7 days before or after another interventional treatment.

In another embodiment of the present invention, the oral composition may further comprise at least one pharmaceutically acceptable excipient known by one skilled in the art.

20 Oral dosage forms of the present invention may comprise suitable diluents, binders, lubricants, antioxidants, disintegrating agents, surfactants, glidants, sweetening agents, coloring agents and coating agents as pharmaceutically acceptable excipients and preferably disintegrant, lubricant and mixture thereof.

the preferred oral dosage forms of the present invention are effervescent tablet, powder or
25 liquid, pellet, granule, solution, suspension or syrup. Specifically, the preferred form of the composition according to the present invention is a liquid oral suspension or in the form of powder to be reconstituted with water. Thus, it can be easily administered to the pediatric population and geriatric population that have swallowing difficulties (dysphagia). Moreover, patients of any age group in the hospital setting, due to the severity of the viral infection or
30 cancer can be easily administered by the composition of the present invention as a liquid

suspension with the help of an injector. So, the preferred form of the present invention would also help increase patient compliance.

Pharmaceutically acceptable diluents of the present invention may be selected from magnesium stearate, lactose, microcrystalline cellulose, starch, pre-gelatinized starch, calcium phosphate,
5 calcium sulphate, calcium carbonate, sodium starch glycolate, mannitol, sorbitol, xylitol, sucrose, maltose, fructose, dextrose and the like or mixtures thereof.

Pharmaceutically acceptable binders of the invention may be selected from starches, natural sugars, corn sweeteners, natural and synthetic gums, cellulose derivatives, gelatin, polyvinylpyrrolidone, polyethylene glycol, waxes, sodium alginate, alcohols, water and the like
10 or mixtures thereof.

Pharmaceutically acceptable lubricants of the present invention may be selected from metallic stearates, metallic lauryl sulfates, fatty acids, fatty acid esters, fatty alcohols, paraffins, hydrogenated vegetable oils, polyethylene glycols, boric acid, polyvinylpyrrolidone, sodium benzoate, sodium acetate, sodium chloride, talk and the like or mixtures thereof.

15 Pharmaceutically acceptable glidants of the present invention may be selected from silicon dioxide, magnesium trisilicate, starch, talc, silicon hydrogel and the like or mixtures thereof.

Pharmaceutically acceptable disintegrating agents of the present invention may be selected from starches, cellulose derivatives, polyvinylpyrrolidone, crospovidone, clays, ion-exchange resins, alginic acid, sodium alginate and the like or mixtures thereof.

20 The preferred antioxidants of the present invention are phenolic antioxidants selected from butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG) or tert-butyl hydroquinone (TBHQ). It is of considerable importance as the addition of a phenolic antioxidant can increase the effectivity of the viral therapy with niclosamide as phenolic antioxidants, especially BHT has anti-viral properties against the replication of RNA viruses,
25 hence is used as a multipurpose ingredient within the formulation.

According to the present invention, the composition includes a phenolic antioxidant selected from butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ).

Sweeteners suitable for inclusion in the present invention may be determined by one skilled in the art including, for example without limitation, both natural and artificial sweeteners such as the representative sweetening agents of intense sweeteners such as sorbitol, sucrose, saccharin such as sodium saccharin, cyclamates such as sodium cyclamates, aspartame, sucralose, 5 thaumatin, acesulfame K, and the like, and sugars such as monosaccharides, disaccharides and polysaccharides. Representative sugars useful in the present invention include, without limitation, xylose, ribose, glucose, mannose, galactose, fructose, dextrose, sucrose, maltose, partially hydrolyzed starch or corn syrup, and sugar alcohols such as sorbitol, xylitol, mannitol, glycerin, etc. and combination thereof. Presently preferred as a sugar sweetener is sucralose. 10 Sugar sweeteners may be replaced or augmented by water-soluble artificial sweeteners, such as the suitable artificial sweeteners previously listed and mixtures thereof. The amount of artificial sweetener used in the composition may vary to provide an appropriate amount of sweetness as determinable by one skilled in the art. Mixtures of sweetening and/or flavoring agents are preferably used.

15 Examples of preservatives suitable for use in the present invention include, for example without limitation, one or more alkyl hydroxybenzoates, such as methyl hydroxybenzoates, ethyl hydroxybenzoates, propyl hydroxybenzoates, butyl hydroxybenzoates and the like. Additional preservatives useful in the present invention include, but are not limited to, sodium benzoate, potassium sorbate, salts of edetate (also known as salts of ethylenediaminetetraacetic acid, or 20 EDTA, such as disodium edetate) and antimicrobial agents including parabens (p-hydroxybenzoic acids esters) such as methyl paraben, ethyl paraben, propylparaben, butylparaben and the like, and combinations thereof. Parabens are preferred, with methyl paraben most preferred for use as preservative ingredients to add to the present pharmaceutical composition, although other pharmaceutically acceptable preservatives may be substituted 25 therefore. Preservative(s) as used in the composition are in an acceptable range.

The composition may also contain a viscosity enhancing agent(s) which include but are not limited to gums; sorbitol; glycerol; polyvinyl alcohol; polyvinyl pyrrolidone; polyethylene oxide; cellulose derivatives, such as hydroxypropylmethylcellulose or a salt thereof, alkyl ether of cellulose, such as methylcellulose, ethylcellulose, hydroxyethylcellulose, 30 hydroxyethylmethylcellose and mixtures thereof. Preferably the viscosity-enhancing agent is hydroxypropylmethylcellulose e.g. (HPMC K4M, HPMC K100 LVP; HPMC K15 MP; HPMC E4 MP; HPMC E10 MP CR).

EXAMPLES

Example 1

		200mg/5mL (in mg)	1 unit (Bottle) in mg	1 batch (10.000 Units) in kg
API	Niclosamide	200	4200	42
Emulsifier	Tween 80	400	8400	84
Co-surfactant	Glycerol	325	6825	68,25
Preservative/anti-oxidant	BHT	10	210	2,1
Dextrin compound	Maltodextrin	100	2100	21
Sweetener	Sucralose	10	210	2,1
Flavouring agent	Vanilla	10	210	2,1
Flavouring agent	Lemon	10	210	2,1
Water qs.	Distilled water	3935	82635	826,35

5 Example 2

		200mg/10mL (in mg)
API	Niclosamide	200
Emulsifier	Tween 80	400
Co-surfactant	Glycerol	500
Preservative/anti-oxidant	BHT	20
Dextrin compound	Maltodextrin	100
Sweetener	Sucralose	20
Flavouring agent	Vanilla	20
Flavouring agent	Lemon	20
Water qs.	Distilled water	8720

Manufacturing Method

- 10 a) Adding niclosamide to Tween 80 and mixing for 20 minutes,
 b) Adding glycerol to the mixture a) and mixing again for 20 minutes,
 c) Adding BHT to the mixture b) and mixing again for 20 minutes,
 d) Adding flavoring agents Vanilla and Lemon to the mixture c) and mixing for 10 minutes,

- e) In order to prepare aqueous phase, mixing maltodextine and sucralose with water for 20 minutes,
- f) Adding aqueous phase to the mixture obtained in step d) and mixing for 60 minutes,
- g) Bottle the final mixture away in dark amber suspension bottles

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HPLC ANALYSIS***Chromatographic System :***

Equipment: High Pressure Liquid chromatography (HPLC)

Column : InertSustain C18 ; 250x4.6 mm, 5 μ m

10 **Flow Rate :** 1.5 mL/min

Wavelength : 230 nm

Injection Volume : 3 μ L

Column Temperature : 25 °C

Sample Temperature : 25 °C

15 **Injection Time :** 7 minutes

Retention Time for Niclosamide $\approx$ 5.3 min

Mobile Phase : Buffer (MPA): Organic (MPB) (30:70 v/v)

Dissolution Medium : pH 6.8 Phosphate Buffer

Volume : 900 mL

20 **Apparatus :** Pedal

Speed : 100 rpm

Time : 60 minutes

RESULTS

Table 1 - Certificate of Analysis

TEST	Specifications	Results
Appearance	Light yellow colored homogeneous suspension	Suitable
Taste and Smell	Characteristic aromatic fragrance, vanilla and lemon flavored suspension	Suitable
Recognition	The retention times of Niclosamide peak obtained from the sample and standard chromatograms in the quantification should be similar.	Suitable
Quantification	180.0 mg/10 mL – 220.0 mg/10 mL	198.8 mg/10 mL
-Niclosamide	(% 90.0 - % 110.0)	(% 99.4)
Related Compounds	Up to % 0.50	% 0.15
Total Impurity		
Dissolution	Min. % 1.00 after 60 minutes	% 1.15
- Niclosamide		
Density	0.90 g/mL – 1.10 g/mL	0.93 g/mL
25°C		
Viscosity	250.0 – 600.0 cP	482.4 cP
25°C (R3 Spindle, 150 RPM)		
pH	5.8 – 6.5	6.26
25°C		
Weight Uniformity	The average weight of 10 bottles should be at least 100 g / bottle	100.2 g/bottle
Average Fill Weight		100.4 g/bottle
Single Fill Weight	Each bottle should be at least 100 g / bottle	

Microbiological Controls	Maximum 1000 cfu / g	< 100 cfu/g
-Total Number of Aerobic Microorganisms (TAMCI)	Up to 100 cfu / g	< 10 cfu/g
Total Mold and Yeast Number (TYMC)	Should not be / 1g	None
Escherichia coli	Should not / 10 g	None
Salmonella spp.	Should not be / 1g	None
-Staphylococcus aureus		

Dissolution Test Results with the formulation of Example 2

Table 2 – Dissolution Results at pH:6.8 Phosphate Buffer

		pH:6.8 Phosphate Buffer		
n	Minutes	API	Sample-1	Sample-2
1	5	0,00	1,24	1,22
2	10	0,00	1,19	1,17
3	15	0,00	1,16	1,13
4	30	0,00	1,09	1,12
5	45	0,05	1,13	1,12
6	60	0,08	1,15	1,14
7	120	0,15	1,07	1,08
8	180	0,22	1,18	1,13
9	240	0,3	1,12	1,12
10	360	0,23	1,11	1,15

Table 3 – Dissolution Results at 0.1 N HCl

n	Minutes	0.1 N HCl		
		API	Sample-1	Sample-2
1	5	0,00	0,42	0,41
2	10	0,00	0,32	0,29
3	15	0,00	0,44	0,43
4	30	0,00	0,43	0,45
5	45	0,05	0,46	0,43
6	60	0,08	0,42	0,40
7	120	0,15	0,39	0,39
8	180	0,22	0,41	0,43
9	240	0,3	0,41	0,39
10	360	0,23	0,29	0,42

Table 4 – Dissolution Results at pH:4.5 Phosphate Buffer

n	Minutes	pH:4.5 Phosphate Buffer		
		API	Sample-1	Sample-2
1	5	0,00	0,42	0,45
2	10	0,00	0,33	0,44
3	15	0,00	0,43	0,43
4	30	0,00	0,44	0,44
5	45	0,05	0,43	0,44
6	60	0,08	0,44	0,43
7	120	0,15	0,41	0,41
8	180	0,22	0,45	0,44
9	240	0,3	0,43	0,41
10	360	0,23	0,44	0,44

5 TOXICITY TEST

In order to calculate the oral lethal dose 50 (Oral median lethal dose, LD50), 6 different doses of niclosamide suspension prepared by according to Example 1 (200mg/5ml) were administered

by oral gavage to 10 male Swiss Albino mice of $30 \text{ g} \pm 3\text{g}$. (OECD Guideline for Testing of Chemicals, No 401: Acute Oral Toxicity, Paris: Organization for Economic Cooperation and Development, 1987) Clinical results and the number of deaths in each group was recorded for the following 24 hours and LD50 was calculated by plotting the dose response curve (DJFinney (1952) Probit Analysis (2nd Ed), Journal of the Institute of Actuaries, 78 (3): 388- 390).

Table 5 – LD50 Values

	Dose - (mg/kg)	Mice Number	Death	Log Dose
Control	0,00	10	0	
2	125,00	10	0	2,097
3	250,00	10	0	2,398
4	500,00	10	0	2,699
5	1000,00	10	0	3,000
6	2000,00	10	1	3,301
7	3000,00	10	9	3,477
8	4000,00	10	10	3,602

**Calculating LD50/LC50 using
Probit Analysis**

C Group	Log10 Dose	Empirical Probits
6	3,30	3,72
7	3,48	5,84
8	3,60	6,28

LD/LC	LD/LC	95% Fiducial CI	
(%)	mg	Lower	Upper
LD46	2410.880	2143.457	2711.667
LD47	2420.496	2152.007	2722.483
LD48	2430.133	2160.574	2733.321

LD49	2439.795	2169.165	2744.189
LD50	2449.490	2177.784	2755.094
LD51	2459.223	2186.438	2766.041
LD52	2469.001	2195.131	2777.039
LD53	2478.831	2203.871	2788.095
LD54	2488.718	2212.661	2799.216
LD55	2498.670	2221.509	2810.410

Accordingly, oral LD50 of niclosamide was calculated as 2450 mg/kg. No apparent symptoms up to 500 mg / kg dose were observed, whereas mice given 1000mg / kg dose had mild stagnation, depression and symptoms of superficial and it was observed that 9 of 10 mices
5 survived by getting rid of the toxic effect of the drug at a dose of 2000 mg/kg.

However, at 3000 mg/kg and 4000 mg/kg doses, in 45-60 minutes after the application, significant depression, difficulty in breathing and abdominal and frequent respiratory symptoms were common. In addition, after ataxia, tremor and severe muscle spasms, deaths of 8 and 9 mices are observed respectively at 3000 mg/kg and 4000 mg/kg doses.

- 10 In addition, it is known that niclosamide has an oral LD50 value of 5000 mg/kg. Therefore, it is considered safe in terms of toxicity. However, the intraperitoneal LD50 dose of niclosamide decreases by 45-250 mg/kg and intravenously by 7.5 mg/kg. Consequently, when absorption of any orally administered niclosamide increases, the blood plasma level and possible toxic effects can be increased in parallel.
- 15 However, in this study, the oral LD50 dose of the niclosamide pharmaceutical composition according to the present invention is 2450 mg/kg which is the half of the LD50 value of niclosamide of the prior art.

The dose with the lowest possible toxicity and the highest effective plasma level is determined by the present invention.

CLAIMS

1. An oral pharmaceutical composition for use in the therapeutic or prophylactic treatment of diseases caused by a RNA virus, wherein the composition comprises niclosamide in an amount of between 40 to 400 mg.
- 5 2. An oral pharmaceutical composition for use according to claim 1, wherein the composition comprises niclosamide in an amount of between 150 to 300 mg.
3. An oral pharmaceutical composition for use according to claim 2, wherein the composition comprises niclosamide in an amount of between 160 to 240 mg.
- 10 4. An oral pharmaceutical composition for use according to any preceding claims, wherein the composition is administered 2 to 4 times daily.
5. An oral pharmaceutical composition for use according to claim 4, wherein the composition is administered 3 times daily.
- 15 6. An oral pharmaceutical composition for use according to any preceding claims, wherein the diseases caused by RNA virus are selected from the group consisting of Ebola virus disease, MERS (MERS-CoV), SARS (SARS-CoV), SARS-CoV-2 (COVID-19) virus disease, Rabies, viral diseases caused by Orthomyxoviridae family virus including influenza A, influenza B, influenza C, influenza D virus disease, hepatitis A, Rhinovirus, Marburg virus disease, hepatitis C, Yellow fever virus disease, Dengue fever virus disease, West Nile virus disease and ZIKA virus disease.
- 20 7. An oral pharmaceutical composition for use according to claim 6, wherein the diseases caused by RNA virus are selected from the group consisting of MERS (MERS-CoV), SARS (SARS-CoV), SARS-CoV-2 (COVID-19) virus disease.
8. An oral pharmaceutical composition for use according to claim 6, wherein the diseases caused by RNA virus are selected from the group consisting of SARS-CoV-2 (COVID-19), influenza A, influenza B and influenza C.
- 25 9. An oral pharmaceutical composition for use according to any preceding claims, wherein the composition further comprises at least one emulsifier.
10. An oral pharmaceutical composition for use according to claim 9, wherein the emulsifier has an HLB value of between 10 and 25.
- 30 11. An oral pharmaceutical composition for use according to claim 9 or 10, wherein the emulsifier is selected from the group consisting of polyoxethylene derivatives, sorbitan esters, polyethylene glycol derivatives and a combination thereof.

12. An oral pharmaceutical composition for use according to any preceding claims, wherein the composition further comprises at least one dextrin compound.
13. An oral pharmaceutical composition for use according to claim 12, wherein the dextrin is selected from the group consisting of β -cyclodextrin, 2-hydroxypropyl- β -cyclodextrin, sulfobutylether β -cyclodextrin sodium salt, randomly methylated β -cyclodextrin, branched β -cyclodextrin and maltodextrin.
14. An oral pharmaceutical composition for use according to any preceding claims, wherein 1% to 6% of niclosamide is released within 30 minutes when subjected to an in vitro dissolution test at 100 rpm in 900 mL of pH 6.8 phosphate buffer and at 37.0°C. $\pm$ 0.5°C.
15. An oral pharmaceutical composition for use according to claim 14, wherein 1% to 6% of niclosamide is released within 60 minutes when subjected to an in vitro dissolution test at 100 rpm in 900 mL of pH 6.8 phosphate buffer and at 37.0°C. $\pm$ 0.5°C.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/TR2021/050689

A. CLASSIFICATION OF SUBJECT MATTER		
A61K 31/167 (2006.01)i; A61K 47/10 (2017.01)i; A61K 47/40 (2006.01)i; A61P 31/14 (2006.01)i		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K; A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPODOC, Google Scholar, PubMed		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2018015153 A1 (UNIV FLORIDA STATE RES FOUND; US HEALTH; UNIV JOHNS HOPKINS) 18 January 2018 (2018-01-18) Abstract; paragraphs 4-5, 115, 121, 123-124, 336- 342	1-6, 9-15 7-8
Y,D	Niclosamide for Mild to Moderate COVID-19 - Niclosamide for Patients with Mild to Moderate Disease from Novel Coronavirus (COVID-19) (NCT04399356) - (First Posted: May 22, 2020 – Last Update Posted: October 28, 2021) (Whole Document)	7-8
A	Huang, L., Yang, M., Yuan, Y., Li, X., & Kuang, E. (2017). Niclosamide inhibits lytic replication of Epstein-Barr virus by disrupting mTOR activation. Antiviral research, 138, 68-78 (Abstract)	1-15
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: “A” document defining the general state of the art which is not considered to be of particular relevance “D” document cited by the applicant in the international application “E” earlier application or patent but published on or after the international filing date “L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) “O” document referring to an oral disclosure, use, exhibition or other means “P” document published prior to the international filing date but later than the priority date claimed “T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention “X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone “Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art “&” document member of the same patent family		
Date of the actual completion of the international search 30 November 2021		Date of mailing of the international search report 30 November 2021
Name and mailing address of the ISA/TR Turkish Patent and Trademark Office (Turkpatent) Hipodrom Caddesi No. 13 06560 Yenimahalle Ankara Turkey Telephone No. (90-312) 303 11 82 Facsimile No. +903123031220		Authorized officer Dr. Ayben Işıl Özdöğün Telephone No. +903123031621

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/TR2021/050689

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2018015153	A1	18 January 2018	WO	2018017426	A1	25 January 2018
				BR	112019000796	A2	24 April 2019
				US	2020164051	A1	28 May 2020
				US	10940188	B2	09 March 2021