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Contents

Weak Membrane Stabilizing Activity of Sydowinin B and Pitholide B

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A B S T R A C T

Background: *There is a dire need for anti-inflammatory agents presently to solve many health challenges. Extracts of endophytic fungi have been implicated a lot in this area. The aim of the study was to test isolated compounds from the extract of Pestalotiopsis clavispora residing in Phoenix reclinata.*
Methods: *Fungal isolation from Phoenix reclinata, cultivation, extraction isolation of compounds, characterisation and membrane stabilisation assay were carried out by standard methods.*
Results and Discussion: *From the extract of P. clavispora, was isolated three compounds which are 7,8-dihydroxanthenone-8-carboxylic acid methyl ester,sydowinin B and pitholide B. The fungus was from the mangrove plant Phoenix reclinata Jacq. (Arecaceae). Sydowinin B and pitholide B exhibited a very weak membrane stabilizing activity of 4.5 and 2% respectively, at 100 µg/mL and 5% at 200 µg/mL for the two compounds.*
Conclusion: *The compounds have not been isolated before from this fungus and they exhibited a very weak membrane stabilizing activity.*

Keywords: Membrane; fungus; inflammatory; red blood cells*.*

1. INTRODUCTION

A number of models have been described and used to evaluate potential anti-inflammatory drugs, some of which include platelet aggregation, cotton pellet granulation in animals and erythrocyte membrane stabilization [1]. Membrane stabilizing evaluation of erythrocytes membrane shown by some agents is a vital in vitro evaluation method for antiinflammatory action [2]. Hypotonicity-induced haemolysis inhibition has been utilised as a measure of the anti-inflammatory activity of various extracts, fractions and isolated compounds from medicinal plants [3]. The plant, Phoenix reclinata, commonly known as Date palm, is widely distributed throughout the mangrove of sub Saharan Africa [4] and it is utilised folklorically for problems associated with erection and male infertility [5]. Extracts of several endophytic fungi have exhibited excellent biological activities. The essence of the work is to test the effect of isolated compounds from Pestalotiopsis clavispora on inflammation.

2. MATERIALS AND METHODS

2.1 Materials

Sephadex LH-20, Silica gel, heparinized syringe, methanol (hplc grade), hexane, ethylacetate.

2.2 Methods

2.2.1 Plant collection

Collection of a few leaf samples of Phoenix reclinata was done at Onne, Rivers State, Nigeria. Dr. Omokafe A. Ugbogu of the Faculty of Agriculture, University of PortHarcourt did the identification. The voucher specimen (NDUP 200) of the authenticated sample was kept and stored at the Herbarium of the Department of Pharmacognosy & Herbal Medicine, Niger Delta University, Wilberforce Island, Nigeria.

2.2.2 Endophytic fungal isolation and its identification

A non-infected leaf of P. reclinata was gently washed in sterilised water without allowing the tissues to squeeze and then disinfected with ethyl alcohol [70% (v/v)] for approximately three minutes and ultimately re-rinsed in sterilised water. Complete disinfection was justified by the application of the sample on malt agar. The sliced leaf samples were then applied to agar incorporated with antibiotics as described earlier by Kjer and co-workers [6]. The plates were thereafter left at 21 - 22°C to allow hyphae growth. Some of the hyphae were subsequently subcultured into fresh agar until pure colonies were established. The colonies were identified using established molecular techniques via DNA extraction, PCR amplication of ITS-1 region and sequencing [6]. The resulting strain coded LNGS2 was thereafter banked at the Institute of Pharmaceutical Biologie and Biotechnologie, Heinrich-Heine University, Duesseldorf, Germany.

2.2.3 Cultivation and extraction of fungus

To the one hundred grams of commercially available rice in a one-litre flask, one hundred and ten millilitres of distilled water was added and subjected to autoclave at 121°C, 2 bar for a third of an hour. After cooling, ten pieces of the fully grown mycelia were gently added to the top of the rice medium in sterile conditions. It was left for a month at room temperature for full growth. The growth was then abruptly terminated using ethyl acetate (about 500ml) which at the same time acted as the solvent of extraction. It was left in this solvent for 11-12 hours after which complete dryness of the extract was achieved in vacuo resulting in a 2.51g of the extract.

2.2.4 Chromatographic purification

The LNG-S2 extract (2.51g) was dispersed in 20 ml of water and then partitioned into normalhexane (70 ml x 2) and methanol/water [(9:1) (70 ml x 2)] and this afforded 1.32, 1.11 and 0.08 g of hexane (LNGS-2'), methanol (LNG-S2 and aqueous (LNGS-2'' fractions, respectively. Vacuum liquid chromatography (VLC) of LNGS-2 (1.3 g) was carried out on silica for TLC. The solvent systems for the elution process were hexane: ethyl acetate in the ratio: of 10:0, 9:1…5:5…. up to 2:8 and this ended up with VLC

fractions. These were eventually bulked and pooled together into four by TLC (n-hexane: ethyl acetate 8/2 and 7/3 and observed in UV light at ʎ 236 and 254 nm. Using HPLC profile and TLC, about 0.53g of the second bulked fraction which was eluted in hexane: ethyl acetate (3:2, 1:1 & 2:3) was taken for further separation on Sephadex LH-20 (DCM / MeOH; 1/1) and this gave 7 subfractions using HPLC for this process. Fractions 1 and 2 were ultimately cleaned on a semi-preparative HPLC (MeOH/0.1% formic acid in water); 0 sec., 10% MeOH; 300 sec., 10% MeOH; 2100 sec., 100% MeOH; 2700 sec., 100% MeOH) to obtain pure compounds coded LNGS2-E1 (1.0 mg) and E2 (5.10 mg). About 200 mg of the third Sephadex fraction was obtained pure and coded LNGS2-E3. Structural elucidation was carried out using NMR (AVANCE DMX 600) and mass spectroscopic (LC-MS Agilent 1100 series) methods.

2.2.5 Membrane stabilization activity

Evaluation of the anti-inflammatory effects of isolated compounds was carried out by in vitro membrane stabilization according to the method described by Awe et al., (2009) [7,8]. The activity could not be carried out for compound 1 due to low yield. Briefly, 5 mL of fresh whole blood was collected into a heparinized syringe, mixed with an equal volume of Alsever's solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42% and distilled water 100 mL).

2.2.6 Hypotonic solution induced haemolysis

Each compound was dissolved in 5 mL distilled water (hypotonic solution) in graded doses (25, 50, 100, 200 µg/mL). An equal volume (1 mL) of the hypotonic solution containing the compound and the blood were separately transferred in triplicate into centrifuge tubes. This was repeated for the isotonic solution containing the compound and distilled water and 100 mg indomethacin served as control. The tubes were gently and slightly shaken for the content to mix properly and incubation was carried out for half an hour at 98.6o F. This was subjected to centrifugation at 1300 gravity for 180 sec. Evaluation of the absorbance of the haemoglobin of the supernatant was performed by using a spectrophotometer (560 nm) by taking the produced haemolysis of distilled water to 100%. The inhibition (%) of the haemolysis was estimated as:

% haemolysis inhibition = 1- [AL2-AL1/AL3-AL1] X100

Where,

AL1 = Value of absorbance of the compound in an isotonic solution

AL2 = Value of absorbance of the test compound in a hypotonic solution

AL3 = Value of absorbance of indomethacin in a hypotonic solution

3. RESULTS

The spectra data from the proton and Carbon –13 nmr are presented in Tables 1 and 2 while the

membrane stability effect is presented in Fig. 1.

Table 1. NMR spectra data of LNGS2-E1 and E2 in 600 MHZ, CD3OD

Position	LNGS2-E1	LNGS2-E2	
	^{1}H -NMR δ (ppm)	^{1}H -NMR δ (ppm)	^{13}C –NMR δ (ppm)
1			163.4
2	6.76 (1H, s)	7.39 (1H, d, J = 9.11 Hz)	110.9
3			151.7
4	6.99 (1 H, s)	7.53 (1H, d, J = 9.12 Hz,)	106.5
4’			157.1
5	6.34, (1H, dd)	6.75 (1H, s, H)	122.2
6	6.47 (1H, dd)	6.99 (1H, s)	140.6
7	5.01 (1H, d)		66.5
8	4.15 (1H, d)		46
8’			110.9
9			183.1
9’			110.9
10’			158.0
11	4.65 (2H, s)	4.68 (2H, s)	66.5
12			171.9
13	3.69 (3H, s)	3.97 (3H, s,)	54.5

Table 2. NMR spectra data of LNGS2-E3 in 600 MHZ, CD3OD

Position	LNGS2-E3	
	^{1}H -NMR δ (ppm)	^{13}C –NMR δ (ppm)
1	8.03 s	155.1
2		
3		166.5
4	7.90 s	107.9
4’		
5		147.3
6		108.6
7		166.7
8		119.8
8’		
9		173
9’		
10’		
11		85
12	4.52 s	69.9
13		122.2
14		18.6
15	9.79 s	187.9
16	1.38 s	24
17		200.9
18	2.84 (m, 2H)	43.7
19	1.63 (m, 2H)	23.8
20	1.32 (m, 8H, H-20-23)	30
21		30.4
22		33.0
23		22.2
24	0.90 (t, 3H)	14

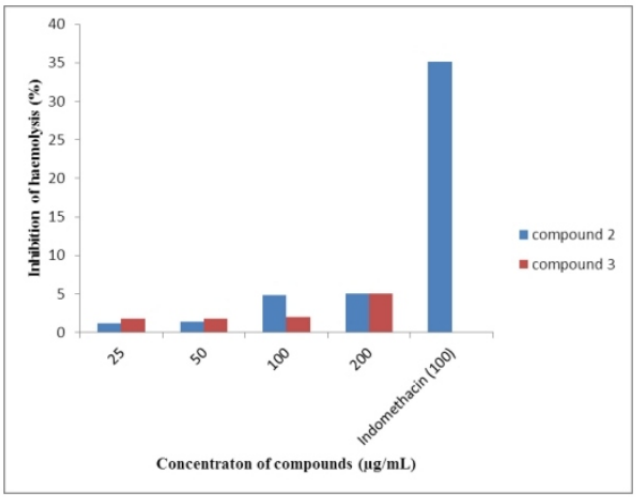
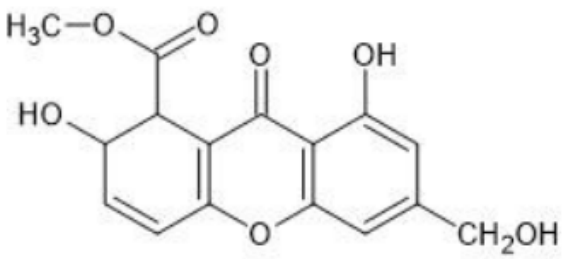


Fig. 1. Membrane stabilising activity of LNGS2-E1 and E2

4. DISCUSSION

4.1 Structural Elucidation

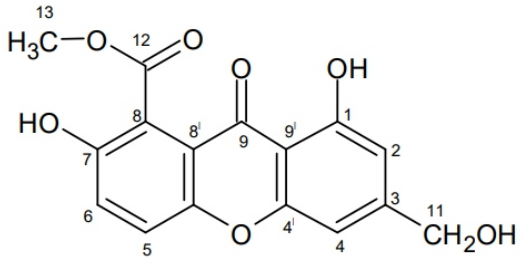
The first compound; LNG-S2-E1 (Fig. 2) was powdery in its physical nature and the colour was yellow, The 1H NMR (600 MHZ, CD3OD) spectrum data are as indicated in Table 1, it was characterised as 7,8-dihydroxanthenone-8-carboxylic acid methyl ester by comparison an earlier study [9].



LNGS-2-EI

Fig. 2. Chemical structure of LNG-S2-E1

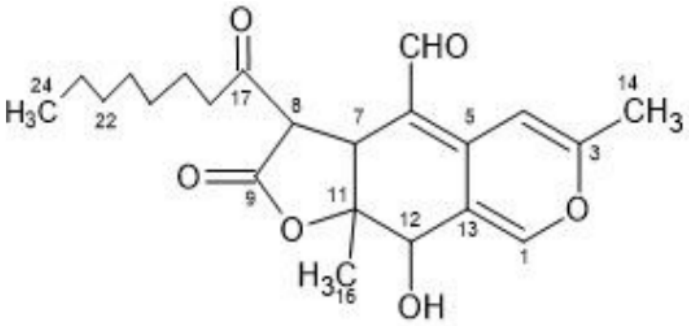
The second compound; LNG-S2-E2 (Fig. 3) was needle-like and yellow. It showed a peak at a retention time; of 22.647 (235 nm). The molecular weight of 316 g/mol was deduced for C16H12O7 as shown by the LC-MS containing quasi-molecular ion peaks at mass/charge of 317.1 and 315.2 which indicated [M+H]+and [MH]-, respectively. The proton and carbon -13 nmrat 600 Megahertz indicated the peaks shown in Table 1. LNG-S2-E2 was characterised to be as Sydowinin B when compared to the literature [10].



LNGS-2-E2

Fig. 3. Chemical structure of LNG-S2-E2

LNG-S2-E3 (Fig. 4) is oily in nature and red in colour. It showed UV absorbance at ʎmax (MeOH) 414.8 nm. As shown by the LC-MS, molecular weight and formula were respectively deduced as 386g/mol and C22H26O6. Quasi-molecular ion peaks at mass/charge 387.2 and 385.3 ([M+H]+and [M-H]-), respectively shown at both positive and negative modes of 386g/mol. Also, The mass/charge peak of 794.9 indicated [2M+Na]+. From the 13C spectrum, three CH3, six CH2, four CH and 9 quaternary carbons which amounted to C22H25 were observed. 1H NMR (600 MHZ, CD3OD) δ (ppm) showed a singlet each with a proton at 8.03, 7.90, 4.52 and 9.79 ppm and a singlet with three protons each of two different positions. Also, two meta protons each of 2.84 and 1.63 were assigned for positions 18 and 19 while others are two protons which are meta each at positions 20 t0 23 and 3 proton peak at position 24 (Table 2). The data extracted from the 13C NMR (600 MHZ, CD3OD) are as also indicated in Table 2. This compound was therefore identified as Pitholide B by comparing it with literature data [11].



LNGS-2-E3

Fig. 4. Chemical structure of LNG-S2-E3

4.2 Membrane Stabilizing Activity

LNG-S2-2 and LNG-S2-3 exhibited a low protective action against red blood cells and lysosome lysis which was influenced by hypotonic solution in rats. The latter is a clear indication of stabilizing action of the membrane. Inflammation may come with age and diseases such as diabetes and cancer among several others [12]. Test for membrane stabilization of red blood corpuscles is gaining popularity in screening for potential anti-inflammatory /agents. Exposure of these blood cells to a medium which is

hypotonic, haemolysis results. Any compounds or agents which can maintain the integrity of the membrane will be suitable anti-inflammatory drugs [7,13,14]. The slight cell membrane stabilizing action exhibited by these compounds is an indication that one of the pathways is mediated via lysosomal membrane stabilization.

5. CONCLUSION

Sydowinin B and pitholide B showed very weak protection against hypotonic solution-induced erythrocyte and lysosome lysis. The weak activity is probably due to de-replication of compounds.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Acute Pancreatitis- A Lesser-known Complication of Celphos Poisoning- A Case Report

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A B S T R A C T

Aluminium phosphide is used to control rodents and pests in grain storage facilities. It is readily available as a fumigant for stored cereal grains and sold under various brand names such as QuickPhos, Salphos and Celphos. It acts by causing cellular hypoxia by its effect on mitochondria, causing inhibition of cytochrome C and by free radicals induced injury with no available antidote. Aluminium phosphide affects various systems, more commonly causing gastrointestinal tract irritation causing marked abdominal pain, shock with refractory hypotension by direct toxicity to heart and various arrythmias, acute respiratory distress syndrome and respiratory failure. Less common features include hepatotoxicity, intravascular haemolysis with methemoglobinemia and/or renal failure. Here we present a case of 24-year-oldnon-alcoholic male, with history of ingestion of approximately6grams of Celphos. At presentation he was drowsy with 2 episodes of vomiting immediately after ingestion of toxin. On subsequent investigations patient was found to have acute pancreatitis which responded to standard fluid resuscitation and acute kidney injury for which 3 episodes of haemodialysis were required. His condition subsequently improved and did not require further dialysis.

There have been only few case reports on aluminium phosphide poisoning primarily causing acute pancreatitis and acute kidney injury. In this case report, we present the case of a young man presented with consumption of aluminium phosphide and then developed acute pancreatitis and acute kidney injury without involvement of other organ systems including heart.

Keywords: Aluminium phosphide; poisoning; acute pancreatitis; acute kidney injur

1. INTRODUCTION

Aluminium Phosphide (AlP) is a commonly used agricultural pesticide. It is cheap, effective, and easily available.Self-poisoning with aluminium phosphide is a common method of suicide in the agricultural community in northern India. The trade name of the fumigant is Celphos, and it comes in the form of dark grey tablets of 3 g each, consisting of aluminium phosphide (56%) and aluminium carbamate (44%). Aluminium phosphide is highly toxic, of lowcost and easily accessible. AlP acts by causing cellular hypoxia by its effect on mitochondria, causing inhibition of cytochrome C and by free radicals induced injury with no available antidote. The mortality rates from acute AlP poisoning (AAlPP) vary from 40 to 80 percent. The actual numbers of cases may be much larger, as less than five percent of those with AAlPP eventually reach a tertiary care centre. Death results from profound shock, myocarditis, and

multi-organ failure.

We report here a case of Aluminium phosphide poisoning presented with features of acute pancreatitis. The incidence of acute pancreatitis without any other organ involvement especially heart and lungs are quite less in fact not known.

2. CASE PRESENTRATION

Twenty-four-year-old, non-alcoholic, married male, presented with alleged history of intake of 6 gram of Celphos (aluminum phosphide) mixed with water around 5pm in the evening. Immediately after ingestion, he developed 2 episodes of non-projectile, non-bilious vomiting containing water and food particles.After this, patient became drowsy and presented to emergency. At presentation his vitals were PR 110/min, BP- 98/68mm hg, RR- 22/min, Spo2-93% on room air and GCS of E1V3M3. Patient was intubated in viewof type 1 respiratory failure. On examination, patient was drowsy, afebrile, pupils were sluggishly reactive to light, abdomen was soft, non-tender with no apparent organomegaly. Initial ECG showed sinus tachycardia (Fig. 1).

Initial arterial blood gas analysis showed normal anion gap metabolic acidosis with respiratory alkalosis [Table 1].

Table 1. ABG showing high anion gap metabolic acidosis with respiratory alkalosis

pH	7.15
PCO2	31
PO2	79
HCO3	11.9
Na+	141.6
K+	4.26
Cl-	113
Lactate	3.5

Onlaboratory evaluation, patient had raised urea of 280.5 mg/dl and creatinine of 7.73 with 24-hour urine output of20ml only. Heunderwent 2 episodes of dialysis subsequently. His initial amylase and lipase levels were 282.8 and 900.8 units/litre which increased rapidly over 24 hours to969 and 4100 units/litrerespectively (Table 2).

USG whole abdomenshowed heterogeneously hypoechoic body and tail of pancreas with its enlargement (1.9 x 2.8 cm) (Fig. 2). Contrast enhanced CT scan was done after 72 hours which showed focal pancreatic body and tail enlargement with surrounding retroperitoneal fat stranding (2.7 x 3.9 x 1.5 cm) (Fig. 3).

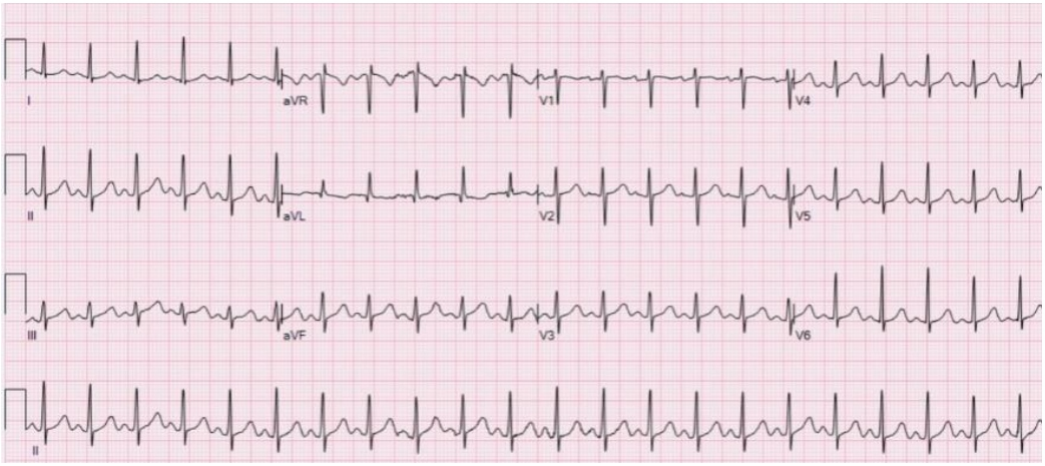

Fig. 1. ECG showing sinus tachycardia

Table 2. Baseline investigations revealing elevated amylase and lipase levels along with deranged Kidney function tests

Hb	9.87	10.5	12.3
TLC	7808	6700	12,300
Platelets	80,000	100,000	135,000
Sodium	152	150	145
Potassium	4.55	3.32	3.61
Calcium	7.96	8.68	7.5
Urea	280.5	171	126
Creatinine	7.23	3.61	2.5
Serum amylase	282.8	969	686
Serum lipase	900.8	4100	2061
Total bilirubin	0.67		
Direct bilirubin	0.43		
AST	53.5		
ALT	47.2		
ALP	119.4		
GGT	89.9		
Serum protein	5.87		
Serum albumin	3.44		
Serum globulin	2.5		

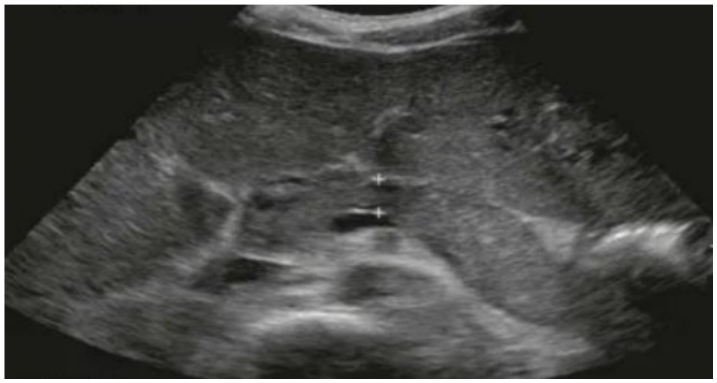

Fig. 2. USG whole abdomen showed heterogeneously hypoechoic body and tail of pancreas with its enlargement (1.9 x 2.8 cm)

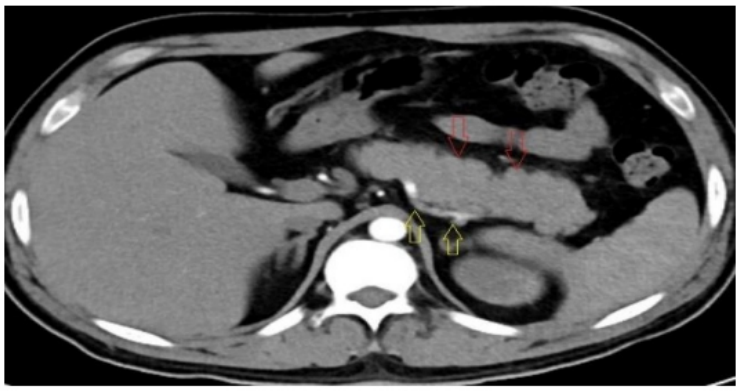

Fig. 3. Contrast enhanced CT scan was done after 72 hours which showed focal pancreatic body and tail enlargement with surrounding retroperitoneal fat stranding (2.7 x 3.9 x 1.5cm)

Fluid resuscitation was started immediately according to protocol and he responded well.Subsequent serum amylase and lipase levels were in decreasing trend.

3. DISCUSSION

"Aluminium phosphide poisoning is the commonest mode of suicide in the agricultural community in northern India. The fatal dose has been reported as 0.5 g for a 70-kg adult with a mean time-interval between poisoning and death being 3 h with a range of 1–48 h." [1] "The signs and symptoms of Acute AlP Poisoning are non specific, dose dependent and evolve with time. After ingestion, toxic features usually develop within a few minutes. The major lethal consequence of AlP ingestion is profound circulatory collapse, secondary to direct effects of toxins on cardiomyocytes, fluid loss, and adrenal gland damage" [2]. "The dominant clinical feature is severe hypotension refractory to dopamine therapy. Other features may include dizziness, fatigue, tightness in the chest, headache, nausea, vomiting, diarrhoea, ataxia, numbness, paraesthesia, tremor, muscle weakness, diplopia, and jaundice. If severe inhalation occurs, the patient may develop ARDS, heart failure, arrhythmias, convulsions, and coma".[2] "AlP can rarely induce complications including hepatitis, acute tubular necrosis, gastroduodenitis, bleeding diathesis, corrosive like esophageal stricture and intravascular" [2].

There are few case reports on agricultural toxin induced pancreatitis, organophosphorus compounds being the commonest cause, but rather a rare complication. It generally shows a subclinical course [3]. "It is mainly caused by acetylcholine release from the pancreatic nerves and the prolonged hyper stimulation of the acinar cells" [4]. "Some patients may also develop serious complications like abscess formations" [4-6].

In 1979, Dressel had described "the first case report of acute pancreatitis with organophosphorus poisoning" [7]. "The patient was a healthy young woman who was brought in a comatosed state. At admission, the serum amylase was found to be 20 times elevated, which had returned to normal by the 4th day" [7]. In 1981, Moore and James had described "another case of acute pancreatitis which had

presented to the hospital in a comatosed state, with signs of organophosphorous poisoning" [8]. It was later confirmed by the low pseudocholinesterase levels. In 1981, Dagli had reported "a case of diazinon poisoning who had developed acute pancreatits. Following this, Dagli and Sheikh studied 75 cases who had been admitted to a general hospital with a definitive history of malathion ingestion" [9,10]. Hyperamylasaemia was found in 47 cases (63%) and in 10 cases (21%), the levels were more than twice the upper limit of the normal levels. The higher level was more than four times the upper limit of the normal level. In 42 cases (89%), the levels had returned to normal within 48 hours of the admission of the patients.In 2013, Venugopal L reported "a case of Toxic Pancreatitis with an Intra-Abdominal Abscess which was Caused by Organophosphate Poisoning, acute pancreatitis was suspected on the third day of his admission, with his serum amylase being 20 times elevated and his ascitic fluid amylase also being very high (46,780 U), which returned to normal in the second week of his admission" [11].

The above-mentioned patient weighing 60 kg consumed two tablets of aluminum phosphide of 3 g each amounting to a highly toxic dose. The United Kingdom guidelines for diagnosis of acute pancreatitis [12] include "a desirable (not mandatory) rise of amylase (or lipase where available) within 48 h of characteristic abdominal pain. A high level of blood sugar, low level of serum calcium, evidence of metabolic acidosis at the time of admission, and raised amylase and lipase levels subsequently with imaging showing oedematous head of pancreas confirmed acute pancreatitis".On the basis of history all common causes of toxin-induced pancreatitis like Ethyl alcohol, Methyl alcohol, Organophosphorus poisoning, Azathioprine, Mercaptopurine, Valproic acid, Didanosine, Corticosteroids, Sulfa drugs, Scorpion venom, Zinc phosphide were ruled out. Radiologically, ultrasound and CECT abdomen helped us to rule out gall stone induced pancreatitis, hypertriglyceridemia, pancreatic duct injury and any other obstructive causes.

"This case characterizes a causative association between acute pancreatitis and aluminum phosphide ingestion, a relationship that has never been observed in the literature available. Given the temporal relationship between ingestion and onset and the absence of any risk factors precluding pancreatitis in this patient, we believe it is reasonable to suggest a probable cause and effect relationship. The speculative mechanism of aluminum phosphide–induced pancreatitis is that, release of phosphine gas results in interaction and inhibition of intracellular enzymes involved in metabolic processes, the most important such enzyme being the cytochrome c oxidase resulting in the release of hydrogen peroxide, superoxide, and other free radicals. Such redox-active compounds are toxic to pancreatic β-cells by lipid peroxidation and other oxidant mechanisms, and oxygen-centred free radicals have been implicated in the induction of pancreatitis" [13,14] "Alternatively, pancreatitis could have resulted from widespread cytokine release, acidosis, and probably ischemia as suggested" by Bogle, et al. [15] In summary, we document a proven case of acute pancreatitis. The patient had no previous medical history or risk factors for the development of acute pancreatitis. Preceding the onset of the attack, he took pellets of aluminium phosphide. Other causes of acute pancreatitis were excluded by clinical history, blood examination, and

abdominal imaging. In the absence of rechallenge, we believe it is probable that aluminium phosphide has a causative link with acute pancreatitis.

4. CONCLUSION

In conclusion, we present a proven case of acute pancreatitis. There was no prior medical history or risk factors for the development of acute pancreatitis in the patient. He had taken aluminium phosphide pellets prior to the attack. Other causes of acute pancreatitis were excluded by clinical history, blood examination, and abdominal imaging. In the absence of re challenge, we believe it is probable that aluminium phosphide has a causative link with acute pancreatitis.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Significance of ISO 10993 Standards in Ensuring Biocompatibility of Medical Devices: A Review

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ABSTRACT

This review paper aims to highlight the crucial role of ISO 10993 standards in ensuring the biocompatibility of medical devices. The use of medical devices has increased rapidly over the years, and it is essential to ensure that these devices do not cause adverse reactions or harm to patients. Biocompatibility testing is a critical aspect of medical device development and is mandated by regulatory bodies worldwide. ISO 10993 standards provide guidelines for evaluating the biocompatibility of medical devices, which includes various tests such as cytotoxicity, sensitization, and irritation. This paper discusses the importance of biocompatibility testing, the different types of ISO 10993 tests, and the challenges faced by the medical device industry in implementing these standards. The review also emphasizes the significance of appropriate material selection, manufacturing processes, and sterilization methods to ensure the biocompatibility of medical devices. Ultimately, the paper highlights the importance of adhering to ISO 10993 standards in designing safe and effective medical devices that benefit patients and healthcare providers alike.

Keywords: Biocompatibility, ISO 10993, medical devices, biocompatibility testing.

1. INTRODUCTION

Medical devices play a vital role in the healthcare industry, providing diagnostic, therapeutic, and monitoring capabilities to patients. However, the use of medical devices also presents potential risks to patients, ranging from minor irritation to severe adverse reactions. Biocompatibility is the ability of a medical device to function without causing any harmful effects on the patient's body. It is a critical factor in the development of medical devices, and regulatory agencies worldwide mandate biocompatibility testing before a medical device can be approved for use. ISO 10993 is a set of international standards that provide guidelines for evaluating the biocompatibility of medical devices. The ISO 10993 standards cover various tests that assess the safety of medical devices, including cytotoxicity, sensitization, and irritation. Adhering to these standards is essential for ensuring that medical devices are safe and effective for patient use. This review paper assesses the significance of ISO 10993 standards in ensuring the biocompatibility of medical devices. The paper discusses the importance of biocompatibility testing, the different types of ISO 10993 tests, and the challenges faced by the medical device industry in implementing these standards. Furthermore, the review emphasizes the significance of appropriate material selection, manufacturing processes, and sterilization methods to ensure the biocompatibility of medical devices. By examining the role of ISO 10993 standards in medical device development, this

review paper aims to provide a comprehensive overview of the importance of biocompatibility testing and its impact on patient safety.

Medical devices have revolutionized healthcare by providing clinicians with new tools and technologies to diagnose and treat a range of conditions. However, as medical devices become increasingly complex, the potential risks associated with their use also increase. Patients may experience adverse reactions ranging from minor skin irritation to severe allergic reactions and even death. Biocompatibility testing is a crucial aspect of medical device development, as it ensures that the device is safe for use within the patient's body.

The International Organization for Standardization (ISO) has developed a set of standards, known as the ISO 10993 series, that provide guidance on how to evaluate the biocompatibility of medical devices. These standards cover various aspects of biocompatibility testing, including the selection of test methods, the interpretation of results, and the safety requirements for medical devices [1,2].

Adherence to the ISO 10993 standards is mandatory for medical device manufacturers who wish to market their products globally. However, implementing these standards can be challenging for manufacturers, as the standards can be complex and require specialized expertise. In addition, the standards are periodically updated to reflect advancements in medical device technology and biocompatibility testing, requiring manufacturers to stay up-todate with the latest guidance.

This review paper aims to provide a comprehensive overview of the importance of ISO 10993 standards in ensuring the biocompatibility of medical devices. The paper highlights the significance of biocompatibility testing and discusses the different types of ISO 10993 tests, including cytotoxicity, sensitization, and irritation testing. Additionally, the review examines the challenges that the medical device industry faces in implementing these standards, such as the need for appropriate material selection, manufacturing processes, and sterilization methods.

Therefore, ensuring the biocompatibility of medical devices is critical for patient safety. Adhering to ISO 10993 standards is a crucial aspect of medical device development, and manufacturers must be aware of the latest guidance to ensure their products are safe and effective for patient use. This review paper provides a valuable resource for clinicians, researchers, and manufacturers in understanding the significance of ISO 10993 standards in the development of medical devices [3].

2. IMPORTANCE OF BIOCOMPATIBILITY IN MEDICAL DEVICES

Biocompatibility is a critical factor in the development and use of medical devices. When a medical device is introduced into the body, it can come into contact with tissues, organs, and bodily fluids. If the device is not biocompatible, it can cause an adverse reaction, such as inflammation, infection, or rejection, which can harm the patient and even be life-threatening [4].

The importance of biocompatibility is especially relevant in the case of implantable medical devices, such as pacemakers, artificial joints, and stents. These devices are designed to remain in the body for

extended periods, which increases the risk of adverse reactions if they are not biocompatible. Even devices that are not implanted, such as surgical instruments and diagnostic tools, can cause harm if they are not biocompatible and come into contact with the patient's body [5].

To ensure that medical devices are biocompatible, manufacturers must conduct rigorous testing to assess their safety and efficacy. This testing is typically performed in accordance with ISO 10993 standards, which provide guidelines for evaluating the biocompatibility of medical devices. By adhering to these standards and conducting thorough biocompatibility testing, manufacturers can help to ensure that their devices are safe and effective for patients [6].

3. POTENTIAL RISKS ASSOCIATED WITH MEDICAL DEVICES

Medical devices can provide significant benefits to patients by diagnosing, treating, or managing medical conditions. However, they can also pose potential risks if they are not designed, manufactured, and used properly. Some of the potential risks associated with medical devices include:

1. Adverse Reactions: Medical devices can cause adverse reactions, such as inflammation, allergic reactions, or infections, if they are not biocompatible or if they come into contact with contaminated surfaces.
2. Mechanical Failure: Medical devices can malfunction due to design flaws, manufacturing defects, or improper use, which can lead to serious harm or even death.
3. Misuse: Medical devices can be misused by patients or healthcare providers, which can result in unintended consequences or complications.
4. Cybersecurity Risks: Medical devices that are connected to the internet or other networks can be vulnerable to cyber attacks, which can compromise patient data or cause the device to malfunction.
5. Lack of Regulation: In some cases, medical devices may not be subject to adequate regulation or oversight, which can result in unsafe or ineffective devices being brought to market

To mitigate these risks, medical device manufacturers must follow strict regulations and guidelines to ensure that their devices are safe, effective, and reliable. Additionally, healthcare providers must be trained on the proper use and maintenance of medical devices to minimize the risk of harm to patients [7,8].

4. ROLE OF ISO 10993 STANDARDS IN ENSURING SAFETY AND EFFICACY

The ISO 10993 series of standards provides a framework for assessing the biocompatibility of medical devices. Biocompatibility is the ability of a medical device to interact with the body without causing adverse reactions such as inflammation, infection, or rejection. The ISO 10993 standards outline a series of tests and evaluation criteria to determine the biocompatibility of a medical device.

The role of ISO 10993 standards is critical in ensuring the safety and efficacy of medical devices. Compliance with these standards is required by regulatory agencies, such as the U.S. Food and Drug

Administration (FDA), for the approval of medical devices for use in patients. By adhering to ISO 10993 standards, manufacturers can demonstrate that their devices have undergone rigorous testing to evaluate their biocompatibility and safety.

The ISO 10993 standards provide guidance on the selection of appropriate tests and evaluation criteria based on the intended use and exposure of the medical device. These tests evaluate various factors, such as cytotoxicity, sensitization, and irritation, to determine the potential risk of adverse reactions. Based on the results of these tests, manufacturers can make informed decisions about the safety and suitability of their devices for use in patients.Therefore, the ISO 10993 standards play a critical role in ensuring the safety and efficacy of medical devices. Compliance with these standards helps to minimize the potential risk of harm to patients and ensures that medical

devices are safe, effective, and reliable [9,10].

5. BIOCOMPATIBILITY TESTING

Biocompatibility testing is a critical component of the medical device development process. The goal of these tests is to evaluate the safety and efficacy of medical devices to ensure that they are biocompatible, meaning that they will notcause harm or adverse reactions when they are used in patients. Biocompatibility testing is typically conducted in accordance with ISO 10993 standards, which provide guidelines for evaluating the biocompatibility of medical devices.

There are several types of biocompatibility tests that can be conducted, depending on the intended use and exposure of the medical device. These tests evaluate various factors, such as cytotoxicity, sensitization, and irritation, to determine the potential risk of adverse reactions.

One commonly used test is the cytotoxicity test, which evaluates the potential of a medical device to cause damage to living cells. This test involves exposing cells to the device or its extract and assessing any changes in cell viability or function. If the device or extract is found to be cytotoxic, it may cause harm to the patient and may not be considered biocompatible.

Another type of biocompatibility test is the sensitization test, which evaluates the potential of a medical device to cause an allergic reaction. This test involves exposing the skin of animals to the device or its extract and assessing any changes in the skin's response. If the device or extract is found to be sensitizing, it may cause an allergic reaction in patients and may not be considered biocompatible.

Other tests, such as the irritation test, systemic toxicity test, and implantation test, may also be conducted depending on the intended use and exposure of the medical device. These tests evaluate various factors, such as inflammation, immune response, and tissue response, to determine the potential risk of adverse reactions.

Overall, biocompatibility testing is a critical component of the medical device development process. By conducting rigorous testing in accordance with ISO 10993 standards, manufacturers can ensure that their devices are safe, effective, and biocompatible for use in patients.

6. SIGNIFICANCE OF BIOCOMPATI BILITY TESTING IN MEDICAL DEVICE DEVELOPMENT

Biocompatibility testing plays a crucial role in the development of medical devices. The goal of these tests is to ensure that the devices are safe and effective, and do not cause any harm or adverse reactions when used in patients. Biocompatibility testing is required by regulatory agencies, such as the U.S. Food and Drug Administration (FDA), to ensure that medical devices meet certain safety standards before they can be approved for use in patients [3,8].

There are several reasons why biocompatibility testing is significant in medical device development. First, it helps to identify potential risks associated with the device. Biocompatibility testing evaluates the potential of a medical device to cause harm or adverse reactions when it comes into contact with the body. This includes assessing the potential for cytotoxicity, sensitization, and irritation, among other factors. If the device is found to be harmful or potentially dangerous, further testing or modifications may be necessary to improve its safety and efficacy.

Second, biocompatibility testing helps to ensure that the device performs as intended. Medical devices are designed to perform specific functions in the body, such as delivering medication or monitoring vital signs. Biocompatibility testing can help to ensure that the device performs these functions accurately and reliably, without causing any harm or adverse reactions.

Third, biocompatibility testing is required by regulatory agencies to ensure that medical devices meet certain safety standards. The FDA requires medical device manufacturers to conduct biocompatibility testing in accordance with ISO 10993 standards to ensure that their devices are safe for use in patients. Compliance with these standards helps to ensure that medical devices are safe, effective, and reliable [3].

Overall, biocompatibility testing is a critical component of the medical device development process. It helps to identify potential risks associated with the device, ensure that the device performs as intended, and ensure that medical devices meet certain safety standards.

By conducting rigorous biocompatibility testing, manufacturers can improve the safety and efficacy of their medical devices and help to ensure that they are safe for use in patients.

7. TYPES OF ISO 10993 TESTS AND THEIR PURPOSE

ISO 10993 is a set of international standards that provide guidelines for evaluating the biocompatibility of medical devices. There are several types of tests included in ISO 10993 that are designed to evaluate different aspects of biocompatibility [1]. The following are some of the most common types of ISO 10993 tests and their purpose:

1. Cytotoxicity testing: This test is used to determine the potential for a medical device or its extracts to cause damage to living cells. It involves exposing cells to the device or its extract and assessing any changes in cell viability or function.

2. Sensitization testing: This test is used to determine the potential for a medical device or its extracts to

to cause an allergic reaction. It involves exposing the skin of animals to the device or its extract and assessing any changes in the skin's response.

3. Irritation and intracutaneous reactivity testing: These tests are used to evaluate the potential for a medical device or its extracts to cause irritation or inflammation. Irritation testing involves exposing the skin of animals to the device or its extract and assessing any changes in the skin's response, while intracutaneous reactivity testing involves injecting the device or its extract into the skin and assessing any changes in the skin's response.

4. Systemic toxicity testing: This test is used to determine the potential for a medical device or its extracts to cause toxic effects throughout the body. It involves exposing animals to the device or its extract and assessing any changes in their physiological functions.

5. Implantation testing: This test is used to evaluate the potential for a medical device or its extracts to cause adverse reactions when implanted in the body. It involves implanting the device or its extract into animals and assessing any changes in the surrounding tissue or systemic response.

Therefore, the different types of ISO 10993 tests are designed to evaluate different aspects of biocompatibility and to ensure that medical devices are safe and effective for use in patients. By conducting these tests, manufacturers can identify potential risks associated with their devices and take steps to improve their safety and efficacy. Different biocompatibility test are given below in Table 1

7.1 Examples of Improved Patient Safety through Biocompatibility Testing

Biocompatibility testing plays a crucial role in ensuring the safety and efficacy of medical devices. Here are some examples of how biocompatibility testing has improved patient safety:

1. Reduced risk of allergic reactions: Sensitization testing can identify potential allergens in medical devices, allowing manufacturers to make necessary changes to reduce the risk of allergic reactions in patients.

2. Improved biocompatibility of implants: Implantation testing can identify potential risks associated with implanted medical devices, such as inflammation or tissue damage. By addressing these issues, manufacturers can improve the biocompatibility of their devices and reduce the risk of adverse reactions in patients.

3. Lower risk of toxicity: Systemic toxicity testing can identify potential toxic effects of medical devices, such as leaching of harmful chemicals into the body. By addressing these issues, manufacturers can reduce the risk of toxicity and improve patient safety.

4. Reduced risk of infection: Biocompatibility testing can identify potential risks of infection associated with medical devices. For example, testing can evaluate the device's ability to resist bacterial growth or assess the potential for biofilm formation. By addressing these issues, manufacturers can reduce the risk of infections associated with their devices.

Overall, biocompatibility testing is essential in ensuring the safety and efficacy of medical devices, and

it has played a significant role in improving patient safety.

Table 1. Biocompatibility tests

Type of Test	Purpose
Cytotoxicity testing	Determine the potential for a medical device or its extracts to cause damage to living cells.
Sensitization testing	Determine the potential for a medical device or its extracts to cause an allergic reaction.
Irritation and intracutaneous reactivity testing	Evaluate the potential for a medical device or its extracts to cause irritation or inflammation.
Systemic toxicity testing	Determine the potential for a medical device or its extracts to cause toxic effects throughout the body.
Implantation testing	Evaluate the potential for a medical device or its extracts to cause adverse reactions when implanted in the body.

8. ISO 10993 STANDARDS

ISO 10993 is a series of international standards that provide guidelines for the biological evaluation of medical devices. The standards aim to ensure the safety and efficacy of medical devices by assessing their potential risks and hazards to human health. The ISO 10993 standards cover a range of tests that evaluate the biocompatibility of medical devices, including tests for cytotoxicity, sensitization, irritation, and systemic toxicity.

The ISO 10993 standards are widely recognized and accepted by regulatory agencies, such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the International Medical Device Regulators Forum (IMDRF). Compliance with these standards is required by many countries around the world for the approval and marketing of medical devices.The ISO 10993 standards are regularly updated and revised to keep up with the latest scientific and technological advancements in the field of medical devices.

The most recent version of the standard, ISO 10993-1:2018, provides a comprehensive framework for the biological evaluation of medical devices and includes guidance on risk assessment, testing strategies, and biocompatibility evaluation. The ISO 10993 standards play a critical role in ensuring the safety and efficacy of medical devices and are an essential component of the regulatory approval process for medical devices worldwide.ISO 10993 parts are described in below given Table 2.

The ISO 10993 series is a set of international standards that provide a framework for the biological evaluation of medical devices. The series consists of 13 individual parts that cover various aspects of biocompatibility testing, including risk assessment, testing strategies, and evaluation of biocompatibility. The standards are designed to ensure the safety and efficacy of medical devices and to protect patients from potential harm caused by exposure to these devices [8].

The ISO 10993 series covers a wide range of topics related to biocompatibility testing, including tests for genotoxicity, carcinogenicity, and reproductive toxicity; selection of tests for interactions with blood; tests for in vitro cytotoxicity; tests for local effects after implantation; tests for irritation and delayed-type hypersensitivity; and tests for systemic toxicity. The series also includes guidance on the ethical use

of animals in testing medical devices and recommendations for reducing animal use and minimizing animal pain and distress.

The ISO 10993 series is widely recognized as the gold standard for biocompatibility testing in the medical device industry. Compliance with these standards is a regulatory requirement in many countries, including the United States, the European Union, and Japan. Adherence to these standards helps to ensure that medical devices are safe and effective for their intended use and that patients are protected frompotential harm caused by exposure to these devices.

Overall, the ISO 10993 series plays a critical role in the development and regulatory approval of medical devices, and adherence to these standards is essential for ensuring patient safety and achieving successful commercialization of medical devices [11].

Table 2. ISO 10993 parts

ISO 10993 Part	Title	Description
Part 1	Evaluation and testing within a risk management process	Provides a framework for the biological evaluation of medical devices and includes guidance on risk assessment, testing strategies, and biocompatibility evaluation.
Part 2	Animal welfare requirements	Provides guidance on the ethical use of animals in testing medical devices and includes recommendations for reducing animal use and minimizing animal pain and distress.
Part 3	Tests for genotoxicity, carcinogenicity, and reproductive toxicity	Describes tests to evaluate the potential for medical devices to cause genotoxicity, carcinogenicity, and reproductive toxicity.
Part 4	Selection of tests for interactions with blood	Provides guidance on the selection of tests to evaluate the interaction of medical devices with blood and includes recommendations for the evaluation of hemocompatibility.
Part 5	Tests for in vitro cytotoxicity	Describes tests to evaluate the potential of medical devices or their extracts to cause damage to living cells.
Part 6	Tests for local effects after implantation	Provides guidance on the evaluation of the potential for medical devices to cause local effects, such as inflammation or tissue damage, when implanted in the body.
Part 7	Ethylene oxide sterilization residuals	Describes methods for the evaluation of residual levels of ethylene oxide and its by-products in medical devices following sterilization.
Part 8	Selection and qualification of reference materials for biological testing	Provides guidance on the selection and qualification of reference materials for use in biological testing of medical devices.
Part 9	Framework for identification and quantification of potential degradation products	Describes a framework for the identification and quantification of degradation products from medical devices.
Part 10	Tests for irritation and delayed-type hypersensitivity	Describes tests to evaluate the potential for medical devices to cause irritation and delayed-type hypersensitivity.
Part 11	Tests for systemic toxicity	Describes tests to evaluate the potential for medical devices or their extracts to cause toxic effects throughout the body.

Part 12	Sample preparation and reference materials	Provides guidance on the preparation of samples for biological testing and the selection and use of reference materials.
Part 13	Identification and quantification of degradation products from polymeric medical devices	Provides guidance on the identification and quantification of degradation products from polymeric medical devices.

9. GUIDANCE PROVIDED BY THE STANDARD

The guidance provided by the ISO 10993 series is essential for ensuring the safety and efficacy of medical devices. By following these standards, manufacturers can ensure that their medical devices are biocompatible and safe for use in patients. This is particularly important given the potential risks associated with medical devices, such as infection, inflammation, and toxicity.

The risk assessment guidance provided by the ISO 10993 series is particularly important, as it allows manufacturers to identify potential risks associated with their devices early in the development process. This, in turn, enables manufacturers to take appropriate measures to mitigate these risks and ensure the safety of their devices.

The selection of tests guidance provided by the ISO 10993 series is also critical, as it helps manufacturers determine which tests are appropriate for their devices. This includes consideration of the type of device, the duration of contact with the body, and the potential routes of exposure.

The guidance on test methods is also essential for ensuring the accuracy and reliability of biocompatibility testing. This includes guidance on sample preparation, test conditions, and interpretation of results.

Overall, the guidance provided by the ISO 10993 series is essential for ensuring the safety and efficacy of medical devices. Compliance with these standards is a regulatory requirement in many countries, and manufacturers who adhere to these standards are more likely to achieve successful regulatory approval of their devices [12,13].

The ISO 10993 series provides guidance on the following aspects of biocompatibility testing:

1. Risk assessment: The standard guidance on how to identify potential risks
associated with medical devices and how to assess the severity and probability of these risks.
2. Selection of tests: The standard outlines a series of tests that can be used to assess the biocompatibility of medical devices. These tests include in vitro tests, animal tests, and clinical tests.
3. Test methods: The standard provides guidance on how to conduct biocompatibility tests and how to interpret the results.
4. Evaluation of biocompatibility: The standard provides guidance on how to evaluate the results of biocompatibility tests and how to determine whether a medical device is safe for use.
5. Biocompatibility data: The standard provides guidance on the type of biocompatibility data that should be included in a medical device submission to regulatory authorities.

6. Ethical considerations: The standard provides guidance on the ethical use of animals in biocompatibility testing and recommendations for reducing animal use and minimizing animal pain and distress.

By providing guidance on these aspects of biocompatibility testing, the ISO 10993 series helps to ensure that medical devices are safe and effective for their intended use and that patients are protected from potential harm caused by exposure to these devices. Compliance with these standards is a regulatory requirement in many countries, including the United States, the European Union, and Japan, and adherence to these standards is essential for achieving successful regulatory approval of medical devices

10. ANALYSIS OF BIOCOMPATIBILITY TESTING METHODS

There are several methods of biocompatibility testing, each with its own strengths and limitations also shown in Table 3.

1. In vitro testing: In vitro testing involves exposing the medical device to cells or tissues in a laboratory setting. This method can be used to assess the cytotoxicity of a device, its ability to induce inflammation, and its potential to cause an immune response. In vitro testing is relatively simple and cost-effective, but it may not accurately reflect the biological environment In vivo. In vitro testing is often used as an initial screening method to evaluate the biocompatibility of a medical device. This method involves exposing cells or tissues to the device, either directly or through extractables or leachables. Common assays used in in vitro testing include the MTT assay, which measures cell viability, and the ELISA assay, which measures cytokine release.

2. In vivo testing: In vivo testing involves implanting the medical device into an animal model to evaluate its biocompatibility. This method is considered the gold standard for biocompatibility testing, as it closely mimics the human biological environment. However, in vivo testing can be expensive and time consuming, and ethical concerns may limit its use. In vivo testing is typically used to confirm the results of in vitro testing and to provide more comprehensive information on the biocompatibility of a medical device. Animal models commonly used for in vivo testing include rodents, rabbits, and dogs.

In vivo testing can evaluate a wide range of endpoints, including tissue reaction, systemic toxicity, and implant performance.

3. Risk assessment: Risk assessment involves evaluating the materials used to manufacture the medical device and determining whether they have a history of safe use in other medical devices. This method can be used to predict the biocompatibility of a device and may be used in conjunction with in vitro and in vivo testing.Risk assessment involves evaluating the materials used to manufacture a medical device and determining whether they have a history of safe use in other medical devices. This can include testing for extractables and leachables, reviewing previous biocompatibility testing data, and assessing the chemical and physical properties of the materials. Risk assessment can be used to predict the

biocompatibility of a device and to guide the selection of appropriate testing methods.

4. Literature review: A literature review involves searching scientific literature for reports on the biocompatibility of similar medical devices. This method can provide valuable information on the potential biocompatibility of a device, but it may not be comprehensive and may not apply to all patient populations.A literature review involves searching scientific literature for reports on the biocompatibility of similar medical devices. This method can provide valuable information on the potential biocompatibility of a device, including information on potential risks and mitigation strategies. However, it may not be comprehensive and may not apply to all patient populations.

5. Computational modeling: Computational modeling involves using computer simulations to predict the biocompatibility of a medical device. This method can be used to evaluate the effects of different materials and designs on biocompatibility, but it may not accurately reflect the complex biological interactions that occur In vivo. Computationalmodeling involves using computer simulations to predict the biocompatibility of a medical device. This can include modeling the interaction between the device and biological tissues, as well as the effects of different materials and designs. Computational modeling can be used to evaluate the effects of different scenarios and to guide the selection of appropriate testing methods.

Therefore, a combination of in vitro and in vivo testing, risk assessment, literature review, and computational modeling may be used to comprehensively evaluate the biocompatibility of a medical device. The choice of testing method(s) will depend on the specific characteristics of the device and the regulatory requirements for approval.

11. CHALLENGES IN IMPLEMENTING ISO 10993 STANDARDS

While the ISO 10993 series provides comprehensive guidance on biocompatibility testing, there are several challenges associated with implementing these standards. Some of the common challenges include:

1. Complexity: The ISO 10993 series is a complex set of standards that requires significant expertise to implement correctly. Manufacturers may struggle to understand the requirements and may require the assistance of specialized consultants or testing labs.

2. Interpretation: The guidance provided by the ISO 10993 series can be open to interpretation, which can lead to variability in testing results between different testing labs. This can make it challenging for manufacturers to compare results and determine whether their devices are safe and effective.

3. Cost: Biocompatibility testing can be expensive, particularly for complex medical devices or those with multiple components. This can be a significant financial burden for manufacturers, particularly for smaller companies or those developing devices for rare diseases.

4. Time-consuming: Biocompatibility testing can be time-consuming, with some tests requiring several

weeks or months to complete. This can delay the development and regulatory approval of medical devices, which can be a significant challenge for companies in highly competitive markets.

5. Limited testing alternatives: Some tests outlined in the ISO 10993 series require the use of animals, which can be ethically problematic and may limit the availability of testing alternatives. This can be a particular challenge for manufacturers developing devices for rare diseases, where animal models may be difficult to obtain.

6. Changes to standards: The ISO 10993 series is regularly updated to reflect changes in scientific understanding and regulatory requirements. Keeping up-to date with these changes can be a significant challenge for manufacturers, particularly for those with limited resources.

Overall, implementing the ISO 10993 standards can be challenging, but it is essential for ensuring the safety and efficacy of medical devices. Manufacturers should work closely with specialized consultants and testing labs to ensure that they are following the latest guidance and that their devices are safe for use in patients [13].

12. FUTURE DEVELOPMENTS IN BIOCOMPATIBILITY TESTING

Biocompatibility testing is a constantly evolving field, and there are several exciting developments that are likely to shape the future of this area. Some of these include:

1. In vitro testing methods: In vitro testing methods have the potential to reduce the reliance on animal testing and offer faster, more cost-effective testing options. Researchers are continually exploring new in vitro methods that can accurately assess the biocompatibility of medical devices.

2. Advanced imaging techniques: Advances in imaging techniques, such as high resolution microscopy and 3D imaging, are likely to improve our understanding of how medical devices interact with the body at the cellular and molecular level.

3. Personalized medicine: The rise of personalized medicine is likely to have an impact on biocompatibility testing, as medical devices become increasingly tailored to individual patients. This may require the development of new testing methods that can accurately assess the biocompatibility of these personalized devices.

4. Integration with regulatory pathways: Biocompatibility testing is a critical component of the regulatory approval process for medical devices. As regulatory requirements continue to evolve, biocompatibility testing is likely to become even more integrated with the regulatory pathway.

5. Risk-based approaches: Risk-based approaches to biocompatibility testing are becoming increasingly popular, allowing manufacturers to focus their testing efforts on the most critical components of their devices. This may involve a greater emphasis on in silico testing and computational modeling.

Overall, the future of biocompatibility testing is likely to be shaped by advances in technology, increased regulatory scrutiny, and a greater focus on personalized medicine. These developments are likely to lead

to more accurate, faster, and cost-effective testing methods, ensuring the safety and efficacy of medical devices for patients [14,15].

13. DISCUSSION

The importance of biocompatibility testing in the medical device industry cannot be overstated. Without proper biocompatibility testing, medical devices may cause harm to patients, and their efficacy may be compromised. Therefore, it is critical for medical device manufacturers to follow ISO 10993 standards to ensure the safety and efficacy of their products.

ISO 10993 standards provide a framework for biocompatibility testing, with each test designed to assess a specific aspect of a device's interaction with the body. By following these standards, manufacturers can ensure that their devices are thoroughly tested and meet the necessary safety requirements before being approved for use in humans.

However, implementing ISO 10993 standards can be challenging, particularly for small and medium-sized companies that may not have the necessary resources or expertise. In addition, as technology and regulatory requirements continue to evolve, manufacturers must stay up-to-date with the latest developments to ensure that their testing methods remain relevant and effective.

Looking to the future, there are several exciting developments in biocompatibility testing, such as the rise of personalized medicine and risk-based approaches. These developments are likely to lead to more accurate, faster, and cost-effective testing methods, ensuring the safety and efficacy of medical devices for patients.

Table 3. Strengths and limitation of biocompatibility testing method

Biocompatibility testing method	Strengths	Limitations
In vitro testing	-Relatively simple and cost-effective -Allows for high-throughput screening -Can evaluate cytotoxicity, inflammation, and immune response	-May not accurately reflect the biological environment in vivo -Cannot evaluate systemic toxicity or implant performance
In vivo testing	-Closely mimics the human biological environment -Can evaluate a wide range of endpoints, including tissue reaction, systemic toxicity, and implant performance	-Expensive and time-consuming -Ethical concerns may limit its use -Animal models may not fully reflect human biology
Risk assessment	-Can predict biocompatibility based on previous data -Can guide the selection of appropriate testing methods -Can be used in conjunction with other methods	-May not accurately reflect the specific device or patient population -Cannot evaluate new materials or designs
Literature review	-Can provide valuable information on potential risks and mitigation strategies -Can guide the selection of appropriate testing methods	-May not be comprehensive -May not apply to all patient populations -May not be up-to-date with current technology and materials
Computational modeling	-Can evaluate the effects of different materials and designs -Can simulate complex biological interactions	-May not accurately reflect the complex biological interactions that occur in vivo -Requires specialized expertise and resources -May not be validated or accepted by regulatory agencies

In conclusion, biocompatibility testing is an essential part of the medical device development process, and ISO 10993 standards provide a vital framework for ensuring patient safety. While implementing these standards can be challenging, staying up-to-date with the latest developments in biocompatibility testing is critical for ensuring the safety and efficacy of medical devices in the future.

14. CONCLUSION

In conclusion, the biocompatibility of medical devices is of critical importance to ensure patient safety and efficacy. The ISO 10993 series of standards provides a framework for biocompatibility testing, which is an essential component of the medical device development process. Through the use of these standards, medical device manufacturers can ensure that their products are thoroughly tested and meet the necessary safety requirements before being approved for use in humans.

While there are challenges in implementing ISO 10993 standards, such as the need for expertise and resources, staying up-to-date with the latest developments in biocompatibility testing is essential for ensuring that medical devices remain safe and effective.

Looking to the future, advances in technology, regulatory requirements, and personalized medicine are likely to shape the future of biocompatibility testing, leading to more accurate, faster, and cost-effective testing methods. As a result, patients can expect a higher level of safety and efficacy from the medical devices that are approved for use.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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Reprofiling Hydrogen Peroxide from Antiseptics to Pyolytics: A Narrative Overview of the History of Inventions in Russia

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A B S T R A C T

Pyolytics are drugs that dissolve thick pus when applied topically. This group of drugs was discovered in early 21st century in Russia as a result of successful repurposing of antiseptics hydrogen peroxide, sodium bicarbonate and sodium chloride from antiseptics to pyolytics. Prior to this watershed event in pharmacy, the problem of effective treatment of purulent diseases had not been solved. The fact is that before that in the treatment of various purulent diseases mainly antiseptics and disinfectants solutions were used, of which hypertonic sodium chloride solution and 3 - 6% hydrogen peroxide solutions took the leading role as "antipurulent" drugs. However, the use of the known antiseptics and disinfectants solutions provided disinfection of the treated surface, but not dissolution of thick pus masses, as the solutions had no effective pyolytic action. Pyolytic activity, i.e. activity of dissolution of thick pus masses, was fantastically increased in hydrogen peroxide solutions only after the possibility of transformation of "old" drugs into "new" drugs by means of artificial changes in physical and chemical properties of their dosage forms was discovered. The greatest (explosive) effect was achieved by developing warm alkaline hydrogen peroxide solutions enriched with oxygen gas under increased pressure. In chronological order, an overview is given of Russian inventions, which formed the basis for the physicochemical repurposing of hydrogen peroxide solutions into pyolytics as well as the basis of temperature and physicochemical pharmacology and pharmaceutics.

Keywords: Development; drugs; physical-chemical properties; purulent diseases; reprofiling; search.

1. INTRODUCTION

In recent years, chemotherapeutic, antiseptic, and disinfectant solutions have been shown to be widely used in the treatment of various purulent diseases throughout the world, but without significant progress

in drug efficacy and without the predictive utility of finding new compounds, especially antibiotics [1,2]. Among them the leaders in application are hypertonic solutions of 2 - 10% sodium chloride and solutions of 3 - 6% hydrogen peroxide [3]. The peculiarity of local application of these and some other drugs is that they are injected into the area of long-term non healing purulent wounds without preheating to body temperature, without prior alkalization and without enrichment with gases [4]. It has been shown that the use of traditional solutions according to the existing medical standards for decades maintained the effectiveness of treatment of long-term non-healing purulent wounds, bedsores and trophic ulcers at the same level [5,6].

In recent years it has been found out that the validity of local application of standard antiseptic solutions according to standard techniques was reduced only to disinfection of the surface treated with them, but not to urgent dissolution of thick purulent masses and their effective removal outside [7]. The fact is that the arsenal of "antipurulent" drugs was not replenished by drugs with pyolytic activity [8]. Moreover, to increase the effectiveness of treatment of purulent wounds with antiseptic and disinfectant solutions, initially some researchers increased the concentration of ingredients in solutions [9]. However, it was soon discovered that increasing the concentration of ingredients in antiseptic solutions increased their physico-chemical aggressive effect on tissues due to an increase in osmotic activity to excessively high values. It was shown that excessively high hypertonic activity of antiseptics increases their disinfecting effect, but at the same time gives these solutions a pronounced local irritating and even cauterizing effect, which can cause death of granulation tissue and delay the process of wound healing. The fact is that many traditional antiseptics and disinfectants have acidic activity, which does not dissolve pus masses, but makes them harder [10,11].

2. METHODS

The information contained in the scientific articles was searched using the following online databases: Google Scholar, Scopus, PubMed, Questel-Orbit, Science Direct, Yandex, and Elibrary. In addition, the information given in the "References" section of the selected scientific articles was analyzed. The information contained in the inventions was searched using Google Patents, EAPATIS, RUPTO, USPTO, Espacenet, PATENTSCOPE, PatSearch, DWPI, and FIIP (RF) databases. Additionally, analogs and prototypes of selected inventions were studied.

The following keywords were used in the search for information: "patent", "invention", "conjunctivitis", "pleurisy", "peritonitis", "arthritis", "panarisis", "abscess", "rhinitis", "tonsilitis", "frontitis", "osteomyelitis", "disease", "wound", "trophic ulcer", "decubitus", "pus", "inflammation", "bronchiectatic disease", "cystic fibrosis", "tuberculosis", "paragonimus", "pulmonary strongyloidosis", "legionary disease", "sputum", "mucus", "pus", "serous fluid", "blood", "catalase enzyme", "treatment", "mucolytics", "expectorants", "pyolytics", "hydrogen peroxide", "sodium

bicarbonate", "solution", "antiseptics" and "disinfectants". Information on scientific articles and inventions was searched without year restrictions. The information that was included in the review was limited to drugs, devices, and medical technologies designed for topical application for the emergency dissolution and removal of thick sputum, mucus, pus, serous fluid, and blood clots. The risk of individual bias in the judgments made was reduced by reliance on the substance of the inventions, since it is the substance of the inventions that is the generally accepted criterion of novelty. The analysis performed included 554 inventions, of which the essence of 39 inventions was included to form an overview.

3. RESULTS

Various purulent diseases are reported in many cases to be long-lasting and dangerous [12]. The danger of these diseases is related to the fact that they can cause sepsis and death [13]. Therefore, the dominant view among medical professionals until now is that purulent diseases are dangerous due to microbial contamination of the blood [14]. On this basis, the treatment of various purulent diseases, including septic wounds, trophic ulcers and bedsores, is based on the local application of chemotherapeutic drugs, antiseptics and disinfectants [15]. Antiseptics are most often used in the treatment of septic diseases, of which solutions of 3-6% hydrogen peroxide and hypertonic solutions of 2-10% sodium chloride take the first place [16].

Nevertheless, the effectiveness of these antiseptics in clearing wounds from pus remains low, so the treatment of purulent diseases is often delayed. In this regard, the search for cheap, affordable and more effective "antipurulent" drugs is urgent. However, the traditional way of searching for and developing new drugs is a very expensive, laborious and long process, which, in addition, does not guarantee success at the end of the research conducted [17].

It is reported that In the late 20th century, a new, very cheap and short way to transform "old" drugs into "new" drugs was proposed by purposefully changing the physical and chemical properties of their dosage forms. Initially, this was done by changing the temperature of drug solutions and tissues during local interaction [18]. To prove the prospects of this direction the authors decided to modernize hydrogen peroxide solutions by replacing their acid activity with alkaline activity, increasing the temperature to +37 - +45 °C and gas content, since hydrogen peroxide belongs to popular over-the-counter and cheap antiseptics and is very often used in the treatment of various purulent wounds [19,20].

Today there is every reason to recognize that the chosen path of modernizing hydrogen peroxide solutions turned out to be the right one, as it resulted in the successful repurposing of hydrogen peroxide from antiseptics to pyolytics [20]. The process of hydrogen peroxide modernization (reprofiling) took about 15 years. During the same period of time more than 30 new drugs incorporating hydrogen peroxide were invented in Russia [21]. Most of them are warm alkaline hydrogen peroxide solutions (WAHPSs) [22]. WAHPSs have been reported to consist of the following main ingredients: water, hydrogen

peroxide and an alkali (preferably sodium bicarbonate) [20]. Many invented drugs have been shown to be able to dissolve thick masses of pus, blood clots, sulfur plugs, tear stones, mucus, sputum, meconium and other thick biological masses containing the enzyme catalase [23,24]. It has been established that WAHPSs, when locally interacting with sputum, mucus, pus, and serous fluid, are capable of exerting a pyolytic, mucolytic, and antihypoxic oxygen-releasing effect [25-29].

The formation of a new direction of search and development of drugs is demonstrated by the chronology of inventions. This can be explained by the fact that all innovative proposals researchers traditionally formalize first in the form of inventions, rather than in the form of scientific articles, since the preliminary open publication of the essence of the created invention excludes obtaining a patent for the invention. We analyzed 561 inventions, of which 44 inventions were used to form an overview. The results of our study showed that more than 30 original hygienic medicines have been invented in recent years for the urgent dissolution, liquefaction and/or bleaching of thick pus, blood clots, stains and crusts, and many other thick or dried biological masses [30]. The vast majority of them were developed in Russia and almost all of them are aimed at hypergaseous, alkaline, hyperosmotic and heating solutions to +37 - +45 °C.

A review of inventions has shown that hydrogen peroxide and sodium bicarbonate have been used to clean surfaces of various objects from various contaminants and for their discoloration in many countries for over 100 years [8,20,21]. However, the first time these ingredients were mixed together to make an alkaline hydrogen peroxide solution was only recently, and it was done in Russia. Other countries continue to use hydrogen peroxide solution with acidic rather than alkaline activity. Because of this, sodium bicarbonate is used, like other alkalis, separately from hydrogen peroxide. Examples of this widespread tradition are inventions for which U.S. patents have been issued, entitled "Hydrogen peroxide-alkali metal bicarbonate acidic bleaching process" (US Patent No. 3017236A, 14.08.1959), "Hydrogen peroxide composition" (US Patent No. 477438, 12.11.1982), "Flavor for peroxide-bicarbonate oral compositions" (US Patent No. 5186926, 03.01.1993) and "Aqueous disinfecting compositions with rapid bactericidal effect" (US Patent No. 20030235623, 21.06.2002). The point is that the above inventions propose a hydrogen peroxide solution with acidic properties, i.e. with a pH value of less than 7.0.

Below is a chronology of inventions that formed the basis of physicochemical pharmacology, the formation of the foundations of which allowed to form a general direction of repurposing hydrogen peroxide solutions from antiseptics to pyolytic, mucolytic, hemolytic and expectorant agents. Historical facts show that the development of physicochemical repurposing of drugs began in Russia in 1986. The fact is that it was then that the first patent for the invention "Method of stopping bleeding" (RU Patent No. 1263248, 15.10.1986), based on the repurposing of 4%potassium chloride solution into a styptic agent by heating it to +39 - +42 °C, was received. Subsequently, changing the activity of drugs by heating

them to a safe level of hyperthermia began to be used more and more often and not only in Russia. Moreover, the foundations of the temperature dependence of pharmacodynamics and pharmacokinetics of drugs (temperature pharmacology) were also laid during this period of time in Russia [31].

The next important step in the development of physico-chemical pharmacology foundations was a patent for the invention "Method for the treatment of long-term non-healing wounds" (RU Patent No 2187287, 20.08.2002). The essence of the invention consists in local application of 3% hydrogen peroxide solution and 2-4% sodium chloride solution heated up to +37 °C, after which the purulent wound area is heated up to the development of persistent tissue hyperemia, but not higher than +42 °C.

Then a patent for the "Floating tablet" invention was received (RU Patent № 2254121, 20.06.2005). The essence of this invention is to artificially increase the airiness of the tablet by creating in it isolated pores filled with air. The porosity of the tablet and its airiness reduces the given specific weight of the tablet, which makes it possible to repurpose all drugs in tablets from "sinking" to "floating" [32].

After that a very important event was a patent for the invention "A method of treatment of tuberculous empyema of the pleura according to N.S.Strelkov" (RU Patent No 2308894, 27.10.2007). The importance of this is related to the fact that euphyllin was first proposed as an "antipurulent" drug (a drug that dissolves thick pus masses). At the same time, it was proposed for the first time to introduce a 24% solution of euphyllin into purulent masses after prior heating it to +42 °C to convert euphyllin from antispasmodics to antiseptics and pyolytics. In this case, a high concentration of the ingredient provided antiseptic properties and high alkaline activity, and hyperthermia potentiated and accelerated the process of alkaline saponification of lipid and protein-lipid complexes that form the basis of purulent masses. In the following years the combination of alkaline activity and local hyperthermia was repeatedly used to develop pyolytic [20].

Then a patent for the invention "Method of peritoneal dialysis" (RU Patent No 2336833,27.10.2008) was received, in which 0.9% sodium chloride isotonic solution was firstly repurposed as an ultrasound contrasting agent due to the preliminary increase of carbon dioxide content under overpressure, which provided visualization of solution flows in a closed abdominal cavity by moving gas bubbles formed during cold boiling.

Practically at the same time there was received a patent for the invention "Hypergassed and hyperosmotic antiseptic preparation" (RU Patent No 2331441, 20.08.2008). This remedy is an aqueous antiseptic solution consisting of 2.7-3.3% hydrogen peroxide, 0.9-10.0% sodium chloride and carbon dioxide to an overpressure of 0.2 ATM at 8°C. The invention provides effective and safe conditions for sanation of purulent wounds, cavities, fistulas, facilitating removal of liquid, viscous and dense biological masses, having a pronounced geyser-like, dehydrating and antiseptic effect.

Also at this time a patent was received for the invention "Method of uterine lavage" (RU Patent No. 2327471, 27.06.2008), the essence of which was washing of the uterine cavity with a solution consisting

of 0.9% sodium chloride and 3% hydrogen peroxide, heated to +42 - +45 °C.

At the same time a patent for the invention "Softening agent for thick and viscous pus" (RU Patent No. 2360685, 10.07.2009) was received. The developed preparation is an aqueous antiseptic solution consisting of 2.7-3.3% hydrogen peroxide and 5.0-10.0% sodium bicarbonate. In it, the peroxide provides a pronounced cold boiling in local interaction with pus, and sodium carbonate provides alkalinity within pH 8.4-8.5 and safe hypertonic activity.

In addition a patent for the invention "Methods of diagnostics and treatment of clotted hemothorax by AY Malchikov" (RU Patent No 2368333, 27.09.2009) was received, in which for rapid and reliable dissolution of blood clots local application of solution containing 5% sodium bicarbonate and 1.5% hydrogen peroxide, heated to a temperature of +37 °C was proposed.

The same year a patent was received for the invention "Method the express remove blood stains from clothes" (RU Patent No. 2371532, 27.10.2009). This invention proposed a hydrogen peroxide solution with a pH greater than 7.0 and a hypoosmolarity of less than 140 milliosmol/L of water at +26 to +42 °C.

At the same time a patent for "Malchikov's method of removing bile calculus" (RU Patent No. 2367375, 20.09.2009) was received. In this invention for destruction of gallstones it is proposed to wet them with warm alkaline solution.

A few years later a patent for the invention "Method and means for removal of sulphur plug" (RU Patent No. 2468776, 27.06.2012) was received. The essence of the present invention is that a solution of 0.3-0.5% hydrogen peroxide and 1.7-2.3% sodium bicarbonate heated to +42 °C is injected into a sulfur plug pricking to complete plug infiltration.

At the same time a patent for the invention "Agent for fistula sanitation in infected pancrenecrosis" was received. (RU Panent No. 2455010, 10.06.2012). The invented drug has special physical and chemical properties that provide inhibition of pancreatic enzymes.

A little later a patent for the invention "E.M.Soikher's hyperoxygenated agent for venous oxygen saturation" (RU Patent No. 2538662, 10.01.2015). The essence of this invention is that the invented drug is an aqueous solution consisting of 0.85% sodium chloride, 0.10% sodium bicarbonate and 0.05-0.29% hydrogen peroxide. The unique formulation provides catalase breakdown of hydrogen peroxide without the formation of gas bubbles, which eliminates blood vessel embolism.

In the same year was received a patent for the invention "Method of maintenance of live fish during transportation and storage" (RU Patent No. 2563151, 20.09.2015). The essence of this invention is the introduction of hydrogen peroxide solution into the water with live fish instead of oxygen.

In parallel, a patent for the invention "Bleach bruising" (RU Patent No. 2539380, 20.01.2015) was received. The essence of this invention is that a solution for intradermal injections was proposed that contains sodium bicarbonate, hydrogen peroxide, EDTA-Na2 and water.

In the following year, several patents were obtained for inventions intended for bleaching blood spots:

"Bleaching agent" (RU Patent No. 2589682, 10.07.2016), "Method for skin bleaching in the bruise area" (RU Patent No. 2586278, 10.06.2016), "Agent for intradermal bleaching of bruises" (RU Patent No. 2573382, 20.01.2016), "Method for skin bleaching in the bruise area" (RU Patent No. 2582215, 20.04.2016), "Agent for increasing resistance to hypoxia" (RU Patent No. 2604129, 10.12.2016) and "Lymphosubstrate for local maintenance of organ and tissue viability under hypoxia and ischemia" (RU Patent No. 2586292, 10.06.2016). The essence of all these inventions is the use of a warm alkaline hydrogen peroxide solution, which provides the release of gaseous oxygen, but excludes vascular gas embolism.

The following year, a patent for the invention "Means for increasing physical endurance" (RU Patent No. 2634271, 24.10.2017) was obtained. The invention is that drinking water includes 7% glucose, 3% hydrogen peroxide and oxygen gas to create an overpressure of 0.2 ATM at +8°C. In addition, this year a patent was received for the invention "Energy drink" (RU Patent No. 2639493, 21.12.2017), intended for enteral feeding of children, made as a sterile solution including glucose, ethyl alcohol, 0,3-0,5% hydrogen peroxide, citric acid, prepared water for injection, containing oxygen gas under excess pressure 0,2 ATM at +8° C. Also this year, a patent for the invention "Carbonated mouthwash" (RU Patent No. 2635992, 17.11.2017), which includes sodium chloride, sodium hydrophosphate, sodium dihydrophosphate, hydrogen peroxide, water and helium gas to create 0.2 ATM overpressure at +8°C was obtained.

At the same time a patent for the invention "Method for emergency bleaching and blood crust removal from skin in place of squeezed out acne" (RU Patent No. 2631593, 25.09.2017), which includes the use of a solution of 3% hydrogen peroxide and 10% sodium bicarbonate was received. Additionally, patents for the inventions "Method for whitening of hematoma under nail" (RU Patent No. 2631592, 25.09.2017), "Method for whitening of bruise under eye" (RU Patent No. 2639283, 20.12.2017) and "Means for intravital skin whitening near blue eyes" (RU Patent No. 2639485, 21.12.2017), which included the local use of a bleach solution at a temperature of +37 -+42 °C, i.e. the use of WAHPSs.

The following year, a patent was obtained for the invention "Bleach bleacher for soaking bandages stuck to the wound" (RU Patent No. 2653465, 08.05.2018), which contains 0.75-1% hydrogen peroxide, 1.2% sodium bicarbonate, 0.5% lidocaine hydrochloride and bidistilled water. The use of the invention provides optimal osmotic, alkaline, buffering, foaming, detergent, anesthetic and bleaching activity of the solution required in wound sanitation for effective and safe softening of the bandage, bloodless and painless removal from the wound and bleaching of the wound and skin around it under surface anesthesia.

In the same year the patents for the inventions "Decolorant of blood" (RU Patent 2647371, 15.03.2018) and "Bleaching cleaner of dentures" (RU Patent No. 2659952, 04.07.2018) were received. The essence of these inventions is that a solution including 3 ± 0.3% hydrogen peroxide and sodium bicarbonate in an

amount ensuring saturation of the solution and preservation of the residue at +42 °C has been proposed to be used as a bleaching agent of blood at skin and textile surfaces. In turn, a solution of 2.0-10.0% sodium bicarbonate and 3±0.3% hydrogen peroxide, which was enriched with oxygen gas at an overpressure of 0.2 ATM at +8°C was proposed for the whitening of dentures. Moreover, it was suggested that the solution be heated to a temperature of +37 - +42 °C before use.

The following year, a patent for the invention "Method of emergency bleaching of skin hematoma under eye" (RU Patent No. 2679334, 07.02.2019) was obtained, in which a solution of 3% hydrogen peroxide and 10% sodium bicarbonate at a temperature of +37 - +42 °C was proposed for bleaching.

Then a patent was obtained for the invention "Method of using a solution to remove plaque with an irrigator" (RU Patent No. 2723138, 09.06.2020). In this invention, a solution of 2.0-10.0% sodium bicarbonate and 2.7-3.3% hydrogen peroxide, which was additionally increase gas argon content at an equilibrium pressure of 3-4 ATM, was first proposed for plaque removal with an irrigator. Moreover, the solution after carbonation was recommended to be stored in a hermetically sealed container and heated to a temperature of +43 to +65 °C before use.

In the same year the patents for the inventions "Gel for children's skin" (RU Patent No. 2713943, 11.02.2020) and "Peeling agent for foot hyperkeratosis" (RU Patent No. 2730451, 24.08.2020) were received. The essence of the invented gel is that it is used heated to +45 °C, consists of water, 0,75-1% hydrogen peroxide, 2% lidocaine hydrochloride and cationic surface active substances in the amount ensuring gel-like consistency at temperature +24-+26 °C, and has alkaline activity with pH value of 8,0-8,5. The essence of the second hygienic preparation is that it is a hypertonic alkaline solution of 3.0-5.0% potassium hydroxide and 0.5-20.0% hydrogen peroxide at pH 13.0-14.0 and osmotic activity 350-560 mosmol/l water heated to +38 -+42 ° C, enriched with oxygen gas to create overpressure of 0.2 ATM. at +8 ° C.

In 2022 a patent for the invention "Dandelion milky juice stains bleaching agent" (RU Patent No. 2765469, 31.01.2022) was received. The bleaching agent relates to household detergents, in particular stain removers, and is intended for urgent dissolution, discoloration and removal of stains of thickened and darkened milky juice of dandelion and other rubbery plants on white clothes. The product is a liquid stain remover consisting of 2 to 4 parts of a solution of 3% hydrogen peroxide and 0.1% detergent, 1 part of a solution of 10% ammonia alcohol and 3 parts of nefras consisting of equal amounts of white spirit and gasoline Kalosha.

In the same year was received a patent for the invention "Glass washing liquid" (RU Patent No. 2763882, 11.01.2022). This glass washer is an aqueous solution containing 0.06-0.5% hydrogen peroxide, 0.1% colorless detergent and 0.08-0.1% ammonia alcohol. Such composition indicates that this glass washer is a variant of alkaline hydrogen peroxide solution. Due to this it provides emergency loosening, softening, discoloration of particles of dung, manure, residuals of insects bodies, turning them into foam

of white color, their urgent removal from the surface of the windshield of the vehicle.

An analysis of the essence of these inventions was performed. The results of the analysis show that all the drugs invented in Russia are solutions that include hydrogen peroxide, sodium bicarbonate and/or sodium chloride and have unique physical and chemical properties [20]. All the above solutions have a nonspecific local action provided by local hyperthermia, moderate alkalinity, osmotic activity, on the one hand, and the enzyme catalase contained in pus masses and other biological tissues, on the other hand. As a result of local application, all alkaline hydrogen peroxide solutions quickly turn these masses into a fluffy white oxygenated foam [22,23,25]. This has been shown to occur due to alkaline saponification of lipid and protein-lipid complexes and a cold boiling process caused by the formation of gas bubbles due to catalase splitting of hydrogen peroxide into oxygen gas and water and/or due to a decrease in the initial overpressure [33,34].

The large number and variety of Russian inventions clearly testify to the continuing progress of WAHPSs development in Russia. In particular, it is reported that aerosols for treatment of respiratory obstruction in purulent obstructive bronchitis (RU patent No. 2735502, 03.11.2020) and for optimization of artificial mechanical ventilation in case of airway obstruction by thick sputum, mucus and pus in the final stage of non-specific bilateral pneumonia in COVID-19 (RU patent No. 2742505, 08.02.2021) were recently invented in Russia [35]. Moreover, these drugs are based on WAHPSs.

4. DISCUSSION

The present review has shown that many purulent diseases have a long lasting character despite the use of the "antipurulent" drugs [12]. It has been shown that in the treatment of various purulent diseases the antiseptics are the leaders among the drugs, and among them are hypertonic solutions of 2 - 10% sodium chloride and solutions of 3 - 6% hydrogen peroxide [36-38]. It has been shown that in the treatment of long-term non-healing purulent wounds these antiseptics are used in accordance with the medical standard, which allows local application of antiseptic solutions at room temperature and with a pH value less than 7.0, that is, with acidic activity [39-41]. At the same time, it was found that cooling from +37 to +25 °C and decrease of pH below 7.0 compacts the colloidal masses. [42]. It was also shown that the injection of cold and acidic solutions of known antiseptics into the area of purulent wounds provides disinfection of the treated surface, but does not dissolve and remove thick and viscous pus [20,43]. It has been reported that the use of known antiseptic solutions according to current medical standards does not provide effective and rapid dissolution and removal of thick and sticky pus in the treatment of long-term non-healing purulent wounds, bedsores and trophic ulcers [44]. It has been established that the reason for the low effectiveness of traditional treatment of bedsores, trophic ulcers and many other purulent diseases, including COVID-19, is the absence of pyolytics in the medical standards of treatment of these diseases [7,20,41]. Meanwhile, the traditional route of finding and developing new antimicrobial drugs

remains a very long and expensive undertaking with a small percentage of new drugs being introduced into medical practice, which has reduced the interest of pharmaceutical companies. Today, experts around the world are looking for an answer to the question, "How can new antimicrobial drugs be developed efficiently, quickly and cheaply?"

It has been shown that the microbiological composition of pus has been well studied, but physical, chemical, physico-chemical and biochemical properties of thick and viscous pus masses in various purulent diseases have not been adequately studied [45,46]. In addition, the mechanisms of local action of antiseptic and disinfectant solutions with purulent masses remain insufficiently studied [43]. Lack of the above information explains why modern medical technologies for treatment of long-term non healing purulent wounds do not provide fast and effective dissolution of dense pus and its removal outward, in particular in tuberculous empyema of the pleura and purulent airway obstruction in COVID-19 [47]. It has been reported that known mucolytic and expectorant drugs also do not provide high speed and efficiency in dissolving and removing thick and sticky pus in various purulent diseases [48]. All this justifies the need to search for new and more effective "antipurulent" drugs [49].

It is shown that in 1986 in Russia the first patent for the invention was received, the essence of which consists in irrigation of the bleeding surface with a repurposed drug, namely - a solution of 4% potassium chloride, but not cold, but heated to a temperature of +39 - +42 ° C. The fact is that a solution of 4% potassium chloride at +39-+42 C has different physical and chemical properties than at room temperature. In particular, local application of a warm solution of 4% potassium chloride causes hyperkaline spasm (contracture) of blood vessels, which helps to stop bleeding. Therefore, safe hyperthermia of the drug solution became the basis for the repurposing of potassium chloride from the pharmacological group "Macro- and microelements" to the group "Blood-stopping drugs". These facts indicate that in this period of history of the development of pharmacology the foundations of temperature pharmacology, temperature conversion of drugs, physical and chemical material science and pharmacy were laid. And it happened in Russia.

A review of the inventions has shown that to date, more than 30 fundamentally new drugs have been invented that differ from all known drugs by their unique physical and chemical properties. The basis of the majority of the invented drugs are warm alkaline hydrogen peroxide solutions (WAHPSs) [8,19,22].

Since such studies are still in their infancy, the main information about them is not found in scientific articles, but in inventions. Therefore, the review presented was based on descriptions of those inventions in which hydrogen peroxide was used. The emphasis was placed on hydrogen peroxide because it is hydrogen peroxide that can urgently decompose into water and molecular oxygen under the influence of the pus mass catalase enzyme. In addition, hydrogen peroxide is an available and over-the counter medication [50,51].

A total of 561 inventions were analyzed, of which 44 inventions were used to form an overview because

only they included hydrogen peroxide and/or were intended for local application for the purpose of dissolving thick colloidal masses containing catalase. Of these, 40 inventions were created in Russia.

The analysis of the contents of the above inventions demonstrated that at the beginning of the 21st century more than 30 drugs were invented in Russia, marking the beginning of hydrogen peroxide re-profiling from antiseptics to pyolytic by purposefully replacing the acid activity of the solutions with alkaline activity within pH 8.4-8.5, heating them to +37 - +45 °C, ensuring isotonic activity and increasing the content of dissolved gases.

It was found that WAHPSs in a single local interaction with pus masses and many other colloidal biological tissues containing the enzyme catalase, very quickly turn them into a fluffy white oxygen foam [19,22]. It was found that when applied topically, WAHPSs have the following effect on pus: they dissolve pus masses due to alkaline saponification of lipid and protein-lipid complexes and simultaneously turn pus into a fluffy foam due to the rapid formation of gas bubbles in the process of cold boiling [8,20,22]. It has been shown that the cold boiling process can be realized by catalase splitting of hydrogen peroxide into water and molecular oxygen, as well as by initially increasing the gas content (oxygen or other gas) in the solution by means of excess pressure [23]. Moreover, it was in Russia that for the first time in the world it was proposed to increase the gas content in antiseptic solutions by creating excess pressure in them and thus turn known medicinal solutions into carbonated solutions with a new mechanism of action and with a new indication for use [4,52,53]. The enrichment of alkaline hydrogen peroxide solutions with oxygen gas or other gases has been shown to increase the pyolytic activity [23]. In addition, local application of WAHPSs has been reported to discolor the skin and nail plate in the area of hematoma and/or bruise, as well as to whiten teeth, dental and ceramic products [8,24,30].

Consequently, the above data indicate the prospects of repurposing hydrogen peroxide from antiseptics to pyolytics due to a targeted change in the physical and chemical properties. It is shown that the advantage and simultaneously the limitation of hydrogen peroxide reprofiling from antiseptics into pyolytics is the local application of drugs and non-specific pharmacological activity due to the original physical and chemical properties of the drugs. The above results convince of the need for further research on the use of targeted changes in the physicochemical properties of hydrogen peroxide for its repurposing from antiseptics to pyolytics. Therefore, it is hoped that more information on the advantages and disadvantages of hydrogen peroxide solutions and other antiseptics with modified physicochemical properties will be obtained in the future.

5. CONCLUSION

Thus, in the late 20th and early 21st centuries in Russia completely new medicines were invented, consisting of "old" medicines, but in new combinations and with new, unique physical and chemical properties, providing a new mechanism of action in local interaction with pus. It is shown that the drugs

of this group are warm alkaline solutions of hydrogen peroxide, which in local interaction with pus masses urgently turn them into a fluffy oxygen foam of white color and due to this clean pus wounds from pathological biological masses and increase the oxygen content in the tissues. Due to this mechanism of local action, warm alkaline solutions of hydrogen peroxide antiseptic are called "Pyolytics" or "Pus Dissolvers".

It has been established that the mechanism of action of pyolytic agents, which are warm alkaline solutions of hydrogen peroxide, is provided, on the one hand, by hyperthermia and alkalinity and, on the other hand, by the presence of catalase enzyme in the purulent masses. The main ingredients of the pyolytic formulations developed for the first time in Russia are indicated. Inventions are listed in chronological order, which allowed to purposefully change physical and chemical properties of hydrogen peroxide solutions, thanks to which hydrogen peroxide has been successfully repurposed from antiseptics to pyolytic. The results obtained give hope for the expansion of the arsenal of "antipurulent" drugs in the near future by including warm alkaline hydrogen peroxide solutions in the role of piolytics. The results show that our narrative review of the history of inventions in Russia indicates that the successful repurposing of hydrogen peroxide from antiseptics to piolytics points to the promise of repurposing "old" drugs into "new" drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Performance of an Antigen Rapid Test Compared to RT-PCR for the Detection of SARS-CoV-2

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ABSTRACT

Background: *Rapid and accurate diagnosis of COVID-19 is critical for the management of patients and to limit the spread of the infection. real-time reverse transcription polymerase chain reaction (RT- PCR) is the gold standard for the diagnosis of SARS-CoV-2 infection, however, it is costly and requires time to obtain a result. A number of alternative rapid tests are available now to provide a faster and more convenient solution for the diagnosis of COVID-19. The aim of this work was to compare the performance of a SARS-Cov-2 Antigen Rapid Test (ART) Cassette to the RT-PCR conventional method.*

Methods: *Two nasopharyngeal swabs were taken from each of the 126 patients included in this study. Of those, 23 were healthy individuals, 9 were confirmed COVID-19 patients and 103 patients from COVID-19 isolation ward in the hospital. For each patient, one sample was processed for RTPCR and a second swab was used on the ART kit.*

Results: *Participants were 57.5% males and 42.5% females. The average age was 54.7 (±14). The QPCR swabs returned 67.9% positivity while the antigen rapid test returned 27.4% positivity. In 56.6% of patients the QPCR results concurred with the rapid test results. Using Fagan nomogram analysis, the 95% confidence interval was (2-20) with a negative likelihood of 0.18. Posterior probability was 0.1. Positive test (blue) prior probability was set at 26%. The 95% confidence interval was (31-41) with a positive likelihood of 1.56. Posterior probability was 0.6.*

Conclusion: *The ART is a useful and efficient test for diagnosing COVID-19, however, QPCR sensitivity is higher. It is recommended to use ART twice for confirming COVID-19 positivity, which will give a statistically more accurate finding.*

Keywords: SARS-CoV-2; diagnosis; rapid test; antigen; RT-PCR.

1. INTRODUCTION

In the month of March 2020, the World Health Organization declared Coronavirus disease 19 (COVID-19) is a global pandemic [1]. This pandemic outbreak was caused by the exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which first appeared in December 2019 in Wuhan,

Hubei Province, China then spread to the rest of the world [2]. However, recently the WHO declared that COVID-19 is no longer a pandemic or an emergency. Infected patients were suffering primarily from acute atypical respiratory symptoms including fever, dry cough, dyspnea, and hypoxia. In addition, other organ systems were also involved [3,4].

Worldwide, approximately 241 million patients were infected with the virus with a total death of around 6.8 million [5]. To control the spread of the infection and react quickly to new cases, faster and cheaper diagnostics are required. Currently, testing approaches fall into two main categories; either nucleic acid or serological [6-8]. Nucleic acid methods directly probe for the viral RNA of a swab taken from the patient throat or nasal cavity [9]. Real time polymerase chain reaction (RT-PCR) a was retained as the goldstandard for clinical diagnosis by the Centers for Disease Control and Prevention (CDC) [10,11].

However, running this method requires the use of special equipment, reagents, and well-trained personnel [12].

The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test (ART) Cassette (INVBIO) is an in vitro immunochromatographic membrane diagnostic test that detect coronavirus antigen using sensitive monoclonal antibodies [13]. Samples can be collected using throat swab, sputum sample, nasal swab, and nasal aspiration [14]. The ART can provide fast (takes less than 10 minutes to develop) and simple alternative to RT-PCR especially for routine screening. In this article, a side-by-side comparison was done to compare the sensitivity of ART to CDCrecommended RT-PCR protocol.

The aim of the work is to evaluate the SARSCov-2 Antigen Rapid Test (ART) Cassette for the detection of SARS CoV-2 antigens in nasopharyngeal swabs, in comparison with the standard RT-PCR technique.

2. MATERIALS AND METHODS

The study was reviewed and approved by the general ethical committee for medical research in the ministry of health (MOH) in Madinah (approval number: IRB48-2021). Written consent was taken from all patients who agreed to participate in the study. All subjects were assigned a study identification number and stayed anonymous and information which identifies patients was not used in this work. The study was performed between May and July 2021.

To study the performance of the rapid antigen test compared to RT-PCR, two nasopharyngeal swabs were taken from 103 COVID-19 hospitalized patients, in addition to 9 positive controls (COVID-19 conformed cases) and 23 negative controls (healthy individuals). On the day when routine swabbing of patients is normally carried out for RT-PCR screening, an additional swab was taken from each patient to be used for the ART (INVBIO, Beijing, China). Results for the first swab were obtained from the hospital record, while the second swab was used on the rapid test onsite.

The kit contains individually sealed strips with two lanes, a bottom small slot for applying the sample and a top lane where the appearance of two bands indicated a positive result, while one band indicated a

positive result, while one band indicated a negative result. To use the kit, the test device was removed from the sterile foil pouch and placed on a clean and level surface. The nasopharyngeal swabs taken from a patient were inserted in the supplied disposable dropper containing 10 drops of the provided extraction buffer, mixed by squeezing and shaking well, before applying three drops onto the trip. Results developed between 2 and 5 minutes. Strips were maintained for further 10 minutes before disposal, to make sure no further changes will occur. Statistical analysis was carried out with a confidence interval (CI) of 95%. The 95% confidence interval was used for the positive and negative likelihood ratios.

3. RESULTS

3.1 Patients QPCR and ART Parallel Tests

Participants in this study were 126 patients, including 23 negative healthy control individuals, 9 positive controls with confirmed COVID-19 positivity by QPCR and 103 patients all from the COVID-19 isolation ward in the local COVID-19 reference hospital. Among those patients, 92.5% were in normal rooms, 4.7% in ICU and 2.8 on mechanical ventilators (MV) in ICU units. Two swabs were taken form each patient in hospital, out of which, one was sent for QPCR and the other for ART. 67.9% of QPCR results were positive while 27.4% of ART were positive (Fig.1). Comparing QPCR results to ART results, when both tests were negative or positive, this was recorded as 'agreement'. Agreement was found in 56% of cases showing a good correlation between the QPCR and ART results (Fig. 1), however, ART was less sensitive.

Fig. 1/A represents the distribution of gender (males/females) participating in the study. Fig. 1/B represents the distribution of patients according to ward and condition; inpatient are patients maintained at isolation rooms at normal room air, ICU represents patients with severe infections maintained in intensive care unit, while MV represents patients with critical conditions, maintained on mechanical ventilators.

Column representation of percentage positive results in all COVID-19 patients in QPCR and ART. The agreement percentage of patients with QPCR and ART identical results is shown in the third column. The percentage included two negative or two positive results for the same patient using QPCR and ART.

3.2 Analysis of Agreement between QPCR and ART

The first hypothesis to be tested was to measure weather QPCR test results is confirmatory of COVID-19 not clinical features. Clinical features included any signs or symptoms of COVID19, which were seen in all hospitalized patients in this study. The likelihood of agreement is shown in Fig. 3 using Fagan nomogram. The figures show the change in posterior probability after the NIRS VOT. The test was considered positive if the delta tissue oxygen index was < 15.2. The positive and negative log-likelihood

ratios were (0.01-1901) and (0.00-1904). Results are shown in Table 1.

Graphical representation showing prior and posterior probability of COVID-19 test results and the likelihood ration. Fagan nomogram negative test (red) prior probability was set at 26%. The 95% confidence interval was (2-20) with a negative likelihood of 0.18. Posterior probability was 0.1. Positive test (blue) prior probability was set at 26%. The 95% confidence interval was (31-41) with a positive likelihood of 1.56. Posterior probability was 0.6.

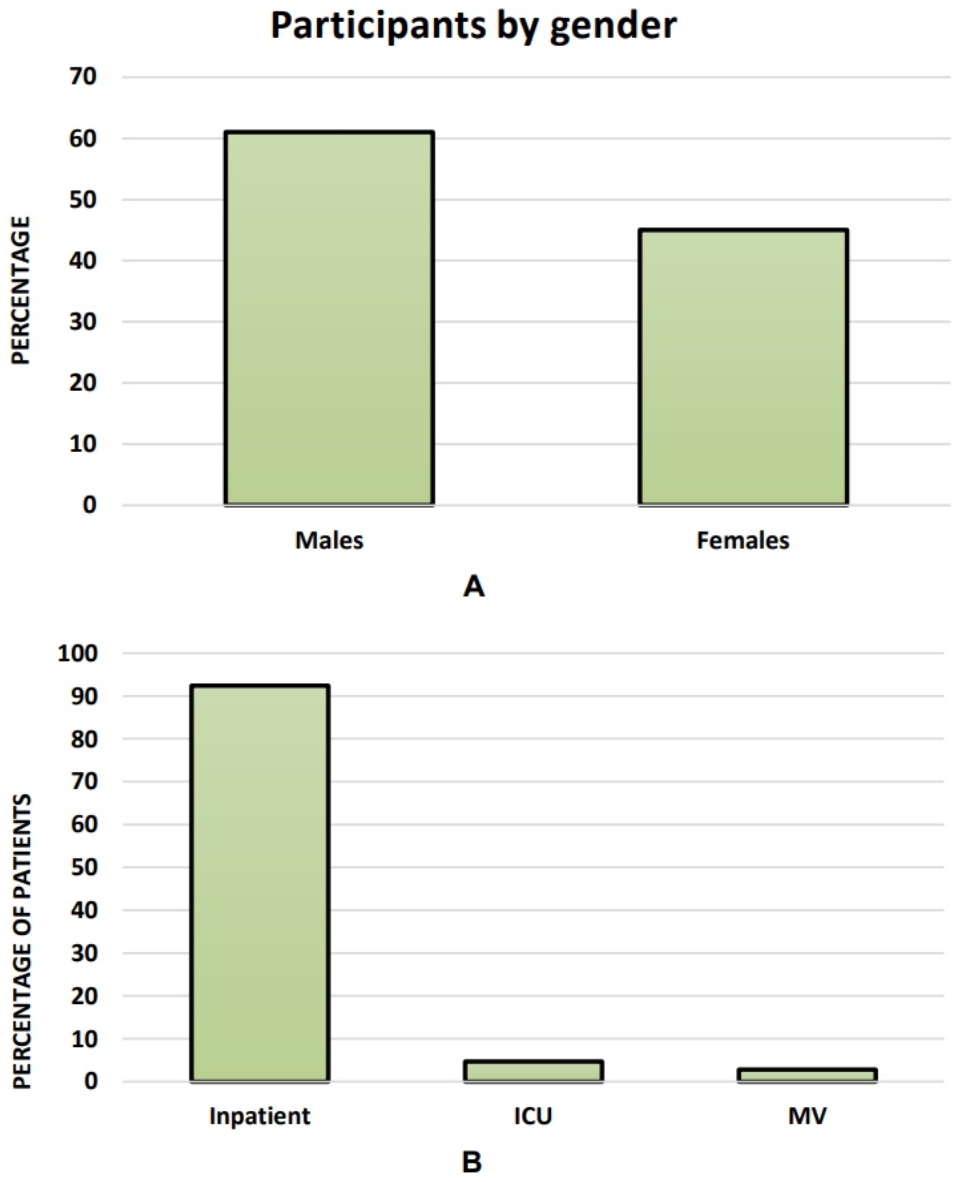

Fig. 1. Participants by gender and percentage of patients

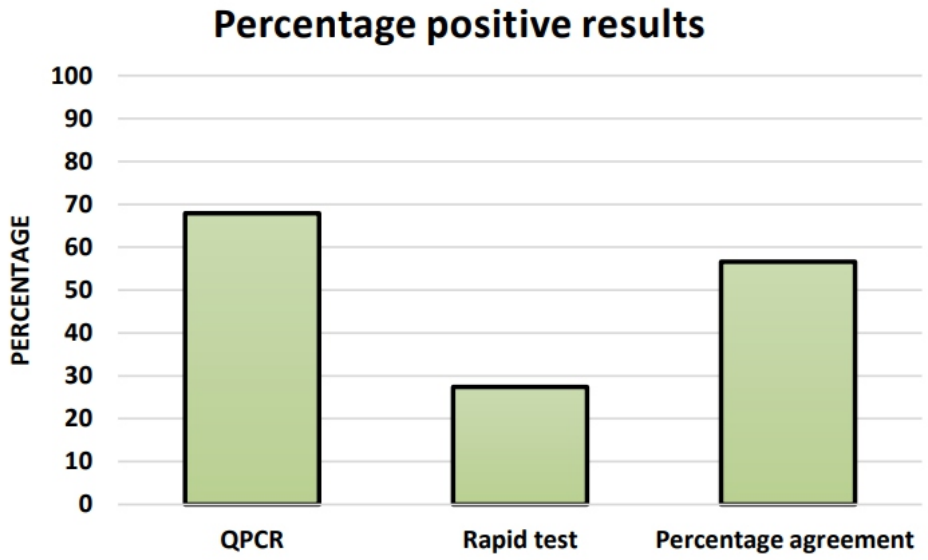

Fig. 2. Positivity of QPCR and ART

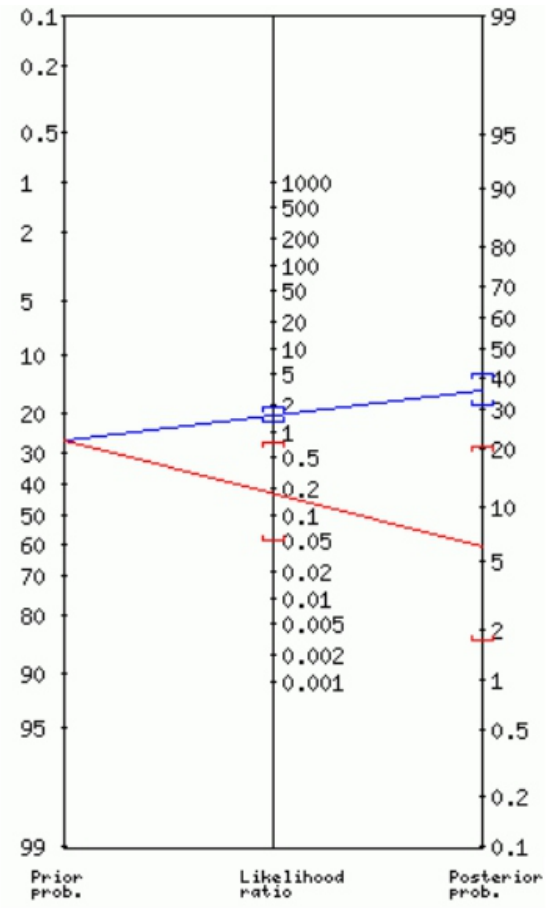

Fig. 3. Fagan nomogram

Table 1. Confidence interval for the positive and negative loglikelihood ratios

Prior probability (odds):	26% (0.4)
Positive Test:	
Positive Likelihood ratio:	1.56
95% confidence interval:	[1.26,1.94
Posterior probability (odd	36% (0.6)
95% confidence interval:	[31%,41%]
Negative Test:	
Negative Likelihood ratio:	0.18
95% confidence interval:	[0.05,0.71]
Posterior probability (odds):	6% (0.1)
95% confidence interval:	[2%,20%]

3.3 Investigating Clinical Features versus QPCR

To investigate whether clinical features will necessarily result in positive QPCR result, the second hypothesis was to determine if clinical features are confirmatory to COVID-19 rather than QPCR tests. Fig. 4 shows the results of the second hypothesis to investigate whether clinical features are confirmatory of COVID-19 infection and not QPCR. It shows a higher percentage within the 95% confidence level.

4. DISCUSSION

SARS-CoV-2 is a member of a large family known as coronavirus causing the COVID19 pandemic. The pandemic is now over as the virus has infected around 841 thousand in Saudi Arabia according to the ministry of health statistics. The gold standard for diagnosis of infected patients with the virus is PCR test, however, faster, and cheaper methods are urgently required to help control the spread of the infection. The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (INVBIO) is an invitro diagnostic test for qualitative detection of coronavirus antigens in nasal Swab and nasal aspirate samples, using the rapid immunochromatographic method [15]. The identification is based on coronavirus antigen specific monoclonal antibody. The assay will provide an easy and fast option especially for healthcare workers routine screening and is a promising tool for combatting the infection [16]. While PCR is currently the gold standard for the detection of the infection, new testing platforms were introduced based on the detection of antigens in nasopharyngeal swabs. Those tests are cheaper and can provide results within minutes. The PCR tests require certified laboratories, expensive equipment, and well trained technicians to operate the instrument. In addition, false negative results have been reported when RT-PCR was used in detecting SARS-CoV-2 [17,18]. These limitations make RT-PCR inappropriate for use when rapid and simple diagnosis is necessary especially in the case of screening healthcare workers, travelers, and patients. Rapid and onsite detection methods can significantly improve the outbreak containment effort [19,20]. Therefore, there is an urgent need for a rapid, simple, sensitive, and accurate test to identify infected patients of SARS-CoV-2 to prevent virus transmission

and to assure timely treatment of patients. The novel coronavirus (SARS-Cov-2) antigen rapid test cassette concept is to employ monoclonal antibodies with specificity for the novel coronavirus antigen [21]. The test is simple and takes 10 minutes to get results. Point-of-care diagnostic tests (POCTs) for detecting viral antigens in clinical samples would be very helpful for the diagnosis of COVID-19 either as massscreening or first aid tests in the emergency room [22].

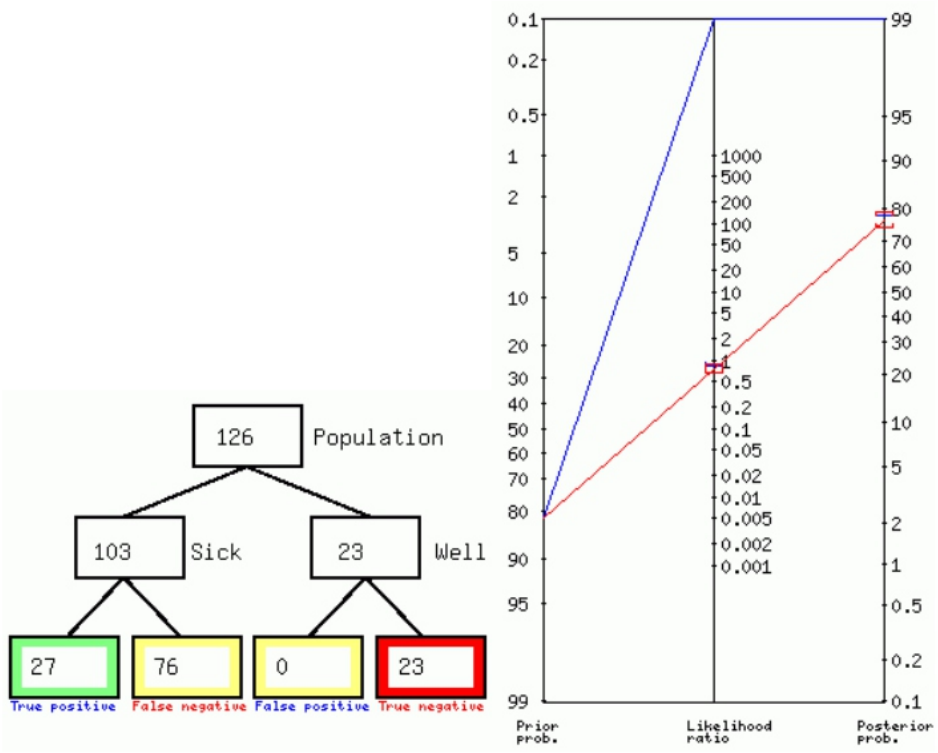

Fig. 4. Results of the second hypothesis to investigate weather clinical features

The SARS-Cov-2 ART kit used to detect SARS CoV-2 antigens in nasopharyngeal swabs employs a rapid immunochromatographic method to identify the viral antigens using specific monoclonal antibodies. The relative sensitivity of the test was around 96.17% and the accuracy 98.79% as reported by the manufacturer. To get precise results using the CDC protocol, the test takes about three hours to complete and costs about $10 [7]. Samples taken from a swab of the nasopharyngeal cavity can harbor approximately 1 million viral particles [23]. In addition, serological tests quantify antibodies in the patient's serum, which tend to be high during the first few days after infection [24].

5. CONCLUSION

The rapid test used in this research has many advantages over PCR due to its has high sensitivity and accuracy. Besides, the cost of the test is much cheaper than PCR and it does not need training to collect or run the sample.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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