BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2022. Vol. **19**(4), p. 1051-1063

Repurposing Over-the-Counter Drugs and an Iron-Chelator as Antibacterial Agents

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http://dx.doi.org/10.13005/bbra/3055

(Received: 01 May 2022; accepted: 26 September 2022)

The conventional drug discovery and development process takes a long time and is not financially viable at times. Repurposing or repositioning existing drugs for treating new diseases seems to be a feasible alternative to this problem. Over-the-counter (OTC) drugs such as Rantac (antacid), Draminate (antiemetic), Diclofenac (painkiller), Sinarest (for respiratory disorders), and Desifer (iron-chelator) were included in this study against eight laboratory cultures. Objective: Repurposing Desifer and the OTC drugs as antibacterial agents. Methods: Aqueous preparations of the OTC drugs and Desifer were checked for their antibacterial activity by the ditch plate method. The Agar cup diffusion method was used to determine the MIC of the individual drugs against gram-positive and gram-negative organisms. The synergistic activity of supernatants of OTC drugs with Desifer was determined using agar cup diffusion and micro broth dilution methods. MTT assay was performed with cell lines to determine anticancer and cytotoxic activity. Results and Discussion: Supernatants of drugs used showed antibacterial activity against at least one laboratory culture used. MIC of OTC drugs decreased to one-fourth of individual MIC when used in combination with Desifer, indicating that Desifer enhanced their inhibitory action. Desifer and Diclofenac exhibit anticancer activity, and low cytotoxicity, therefore could be good candidates as chemotherapeutic agents. Conclusion: A combination of the drugs such as Diclofenac and Desifer could be an effective alternative therapy to treat bacterial infections. With emerging drug resistance, Desifer with OTC drugs proves to be a good strategy to enhance the effectiveness of antibacterial drugs.

Keywords: Desifer; OTC drugs; Repurposing; Siderophore.

While a wide range of bacteria is inhibited by currently available antibiotics, many bacteria have been found to acquire resistance against the available antibiotics. Infectious diseases such as HIV/AIDS, tuberculosis, malaria, and influenza remain a global health concern.[1] Therefore, there is an urgent need to find novel and cheap antibiotics.[2] Developing new drugs using conventional drug discovery processes requires time and finance. According to reports in the period from 1995 to 2001, no new drug candidates were developed by Pfizer, GlaxoSmithKline, and AstraZeneca[3-5], highlighting the challenge of drug discovery and development.

A solution to this issue is the repurposing of existing drugs for treating conditions other than the conditions they are normally used for.[6] Repurposing is an alternative to conventional

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drug discovery and can be used to identify further applications of existing drugs.[7-11] The best example of drug repurposing is the repurposing of thalidomide. Thalidomide was developed as a sedative-hypnotic agent against nausea and morning sickness in pregnant women in the 1950s. However, its use was later prohibited due to its teratogenic and anti-angiogenic effects.[12,13] Although, further research found the drug to be effective in the treatment of multiple myeloma and other related malignancies.[14,15] Currently, it is an FDA-approved drug for multiple myeloma.[16]

Repurposing of commonly used or over-the-counter (OTC) drugs has already been attempted. For example, non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen have been recently proven to have anti-cryptococcal activity as an off-target effect. [17] In addition, many OTC drugs such as aspirin, rapamycin, minocycline, celecoxib, valproic acid, and metformin have been reported to demonstrate anticancer activity.[18] Furthermore, Viagra (sildenafil citrate) was originally used for chest pain and later reprofiled for male infertility. [19] The most recent addition in the repurposing of drugs is for the COVID-19 infections where Remdesivir and Chloroquine were used against the novel coronavirus.[20,21]

Deferoxamine B is an FDA-approved drug for the treatment of iron overload in thalassemia patients.[22-25] It was found to demonstrate anti-tuberculosis activity and thus, could be used as an alternative therapy for treating infections caused by MDR *Mycobacterium tuberculosis* strains.[26]

In the present study, we evaluated OTC drugs including diclofenac (NSAID), dimenhydrinate (antiemetic, antihistamine, and anticholinergic agent), Sinarest (Combination medicine), Rantac (Antacid), and an iron chelator Desifer for their antibacterial and anticancer activity as well as cytotoxicity. OTC drugs were evaluated alone and in combination with Desifer. Drugs included in the study are FDA-approved and have a long record of safety in patients.

The development of such an alternative therapy will be extremely useful in cancer chemotherapy or the treatment of infections caused by drug-resistant pathogenic bacteria.

MATERIALS AND METHODS

Determination of antibacterial activity

Determination of antibacterial activity of Draminate, Diclofenac, Rantac, Sinarest, and Desifer by ditch plate method

Tablet formulations of Draminate (Dimenhydrinate), Diclofenac(Diclofenac sodium IP), Rantac(Ranitidine hydrochloride IP), Sinarest (Paracetamol IP), and Desifer (Deferasirox) were used Qualitative determination of the antibacterial activity of these drugs was conducted using the agar ditch method according to the protocol given by Rice et al.[27]

The powdered drugs (Draminate, Diclofenac, Rantac, and Sinarest) were poured into a tube containing 4ml of molten Nutrient agar and this mixture was poured into the ditch. Two concentrations of each drug were used. The following 8 laboratory test cultures were used: Gram-negative organisms – *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa* , and gram-positive organisms – *Staphylococcus aureus, Mycobacterium smegmatis, Streptococcus pyogenes,* and *Corynebacterium diphtheriae.* Four test cultures were streaked on each plate. The plates were incubated at 37!for 24 hours.

Determination of Antimicrobial activity of Drug pellet and Drug supernatant

The drugs Draminate, Diclofenac, Rantac, Sinarest, and Desifer (desferri-form), were dissolved in water, this suspension was then centrifuged at 10,000 rpm for 10 minutes. Next, the supernatant was collected and sterilized using a 0.2µm membrane syringe filter. Sterile nutrient agar plates were swabbed with test culture suspension (*E.coli, K. pneumoniae, S. typhi, P. aeruginosa, S. aureus, M. smegmatis, S.pyogenes,* and *C. diphtheriae*), the turbidity of which was adjusted to 0.5 MacFarland standard . The supernatant and pellet were spotted onto these plates and incubated at 37! for 24 hours. The plates were checked for a zone of inhibition after incubation.

Determination of the inhibitory effect of different concentrations of OTC drugs (Draminate, Diclofenac, Rantac, Sinarest), and the iron-chelator Desifer by agar cup diffusion method

The Agar cup diffusion method was performed as per the protocol given by Rose and

Miller.[28]Culture suspensions (turbidity adjusted to 0.5 MacFarland standard) of the eight laboratory cultures (*E. coli, S. aureus, M. smegmatis, S. pyogenes, C. diphtheriae, K. pneumoniae, S. typhi,* and *P. aeruginosa*) were pour plated using sterile iron-deficient Mueller Hinton (MH) agar medium. Aqueous supernatants(100µl aliquots) of Diclofenac (0.5, 1, 5, 10, 15, and 20 mg/ml), Draminate (15, 20, 25, 30, 35, 40, 45, and 50 mg/ml), Rantac (25, 50, 75, and 100 mg/ml), Sinarest (125 and 250 mg/ml) and Desifer (0.5, 1,5,15,25, and 40 mg/ml) were added in cups . The plates were incubated at 37! for 24 hours and checked for a zone of inhibition.

Determination of the antibacterial activity of Draminate, Diclofenac, Rantac, and Sinarest each in combination with Desifer

Agar cup diffusion method

Culture suspensions (turbidity adjusted to 0.5 MacFarland standard) of the eight laboratory cultures (*E. coli, K. pneumoniae, S. typhi, P. aeruginosa, S.aureus, M. smegmatis, S. pyogenes,* and *C. diphtheriae*) were seeded in sterile iron deficient MH agar medium. Cups were bored in these plates. Aqueous supernatants of the 4 drugs (Draminate, Diclofenac, Rantac, and Sinarest) were diluted and 50µl of these dilutions were added in combination with 50µl of Desifer (40 mg/ml). Desifer-only controls were applied by adding 50 µl each of Desifer stock and distilled water to the cup. The plates were incubated at 37 ! for 24 hours and checked for a zone of inhibition.

Micro broth dilution method

Culture suspensions (turbidity adjusted to 0.5 MacFarland standard) of the 8 laboratory cultures (*E. coli, K. pneumoniae, S. typhi, P. aeruginosa, S.aureus, M. smegmatis, S. pyogenes,* and*C. diphtheriae*) were prepared in sterile iron-deficient double-strength MH (Mueller-Hinton) broth. These suspensions (100 µl aliquots) were added to a sterile 96-well microtiter plate. Dilutions of the four drugs (Draminate, Diclofenac, Rantac, and Sinarest) (50µl) were added individually and in combination with 50µl of Desifer (40mg/ml). Drug-only controls were applied by adding 50 µl each of drug stock and distilled water in the culture-containing wells. Appropriate negative controls were used. The microtiter plate was incubated at 37! for 24 hours. Furthermore, a growth curve was obtained by measuring the turbidity at 30-minute intervals for 24 hours. Absorbance was measured at 600nm using a Microplate reader (Epoch 2, BioTek). Bactericidal activity of these combinations was determined by spotting 10µl samples from wells showing no growth onto sterile MH agar plates, which were incubated at 37! for 24 hours.

Determination of anticancer and cytotoxic activity

Anticancer activity and cytotoxicity of the drugs under study were checked (individually and in combination with Desifer) against human lung cancer cell line A549 and human embryonic kidney

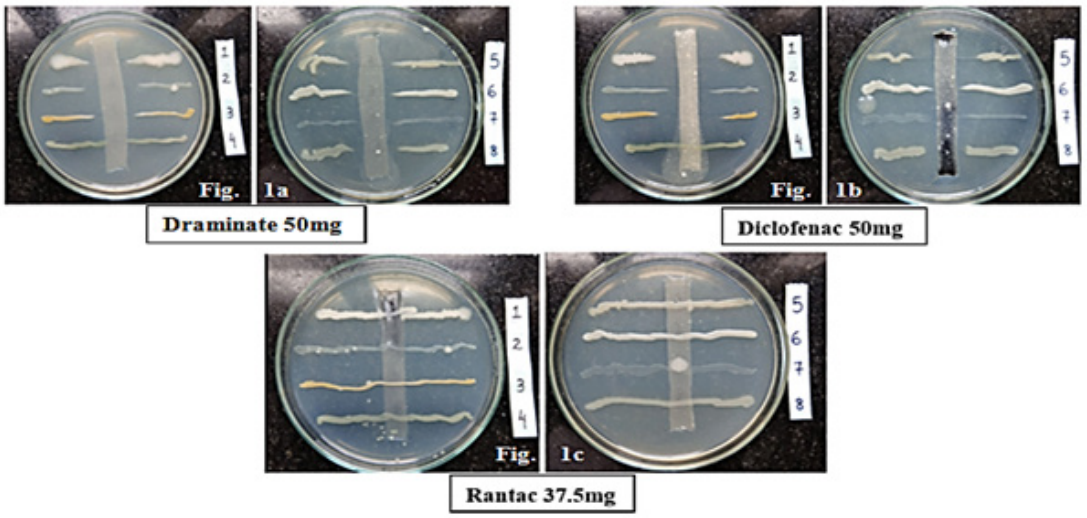

Fig. 1a,1b, and 1c. Represent the antimicrobial activity of Draminate (50mg), Diclofenac (50mg), and Rantac (37.5mg), respectively, against laboratory strains

(HEK293) cell lines, respectively. MTT assay was performed for checking the anticancer activity and cytotoxicity of these drugs. Absorbance was measured at 590 nm using an Epoch 2 plate reader and percentage survival rates were calculated using Sigma Stat Software.

Analysis of aqueous supernatants of Draminate, Diclofenac, Rantac, Sinarest, and Desifer

Detection of active components in aqueous supernatants of the selected drugs using Thin Layer Chromatography

A chromatography chamber was saturated with Methanol: Acetone (8.5:6.5) solvent vapors. A silica gel plate was loaded with aqueous supernatants of Draminate, Diclofenac, Rantac, Sinarest, and Desifer, this was placed in the saturated chamber and allowed to run until the solvent front had reached an appropriate length. The chromatogram was baked at 60! for 10 minutes and then developed using saturated iodine vapors.

Qualitative chrome azurol S (CAS) assay for detection of active siderophores in the aqueous supernatant of Desifer

Chrome azurol S (CAS)assay is the universal method for detection of iron-chelators/ siderophores and was used to detect the presence of an active component (deferasirox) in the aqueous supernatant of Desifer. CAS dye changes from blue to orange in the presence of iron chelators.[29]

Quantitative CAS assay for measuring the concentration of active siderophores aqueous supernatant of Desifer

CAS dye was used for quantitative estimation of deferasirox in the aqueous supernatant

Fig. 2. Shows examples of inhibition zones of *S.aureus* and *E.coli* by different concentrations of Draminate

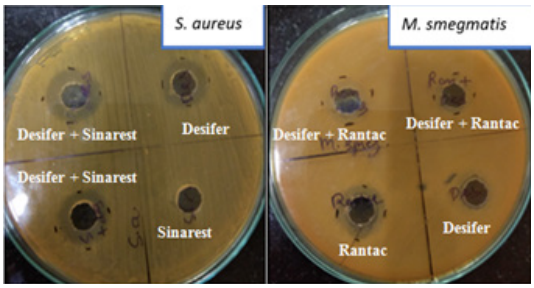

Fig. 3. Shows representative examples of zones of inhibition of *S.aureus*caused by Desifer alone and in combination with Sinarest as well as no inhibition by Sinarest alone, zone of inhibition of *M.smegmatis* caused by Desifer and Rantac alone as well as in combination.

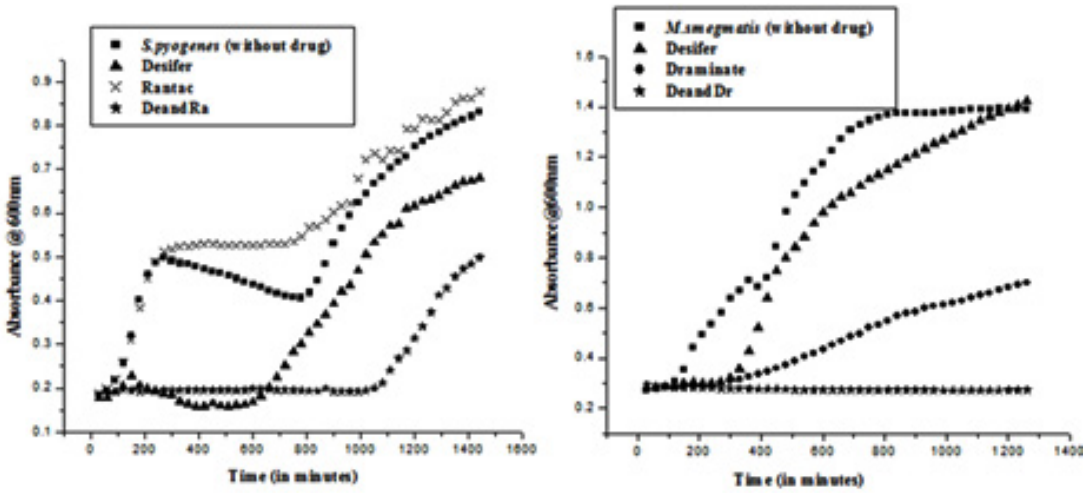

Fig. 4(a). Bacteriostatic activity of Rantac and Draminate each in combination with Desifer on the growth of *S. pyogenes* and *M. smegmatis,* respectively.

of Desifer. DFO-B (injectible) was used as the standard iron-chelator. A standard curve of DFO-B concentration (mg/ml) v/s absorbance at 630 nm was prepared. Deferasirox concentration in the aqueous supernatant was determined using this standard curve. To 100 µl aliquots of drug different drug concentrations, 10 µl CAS dye was added. Absorbance was determined using an Epoch 2 plate reader.

RESULTS

Determination of antibacterial activity

Determination of the antibacterial activity of Draminate, Diclofenac, Rantac, Sinarest, and Desifer by ditch plate method

Draminate and Diclofenac showed antibacterial activity against all laboratory cultures except *P. aeruginosa.* However, selected concentrations of Rantac and Sinarest did not

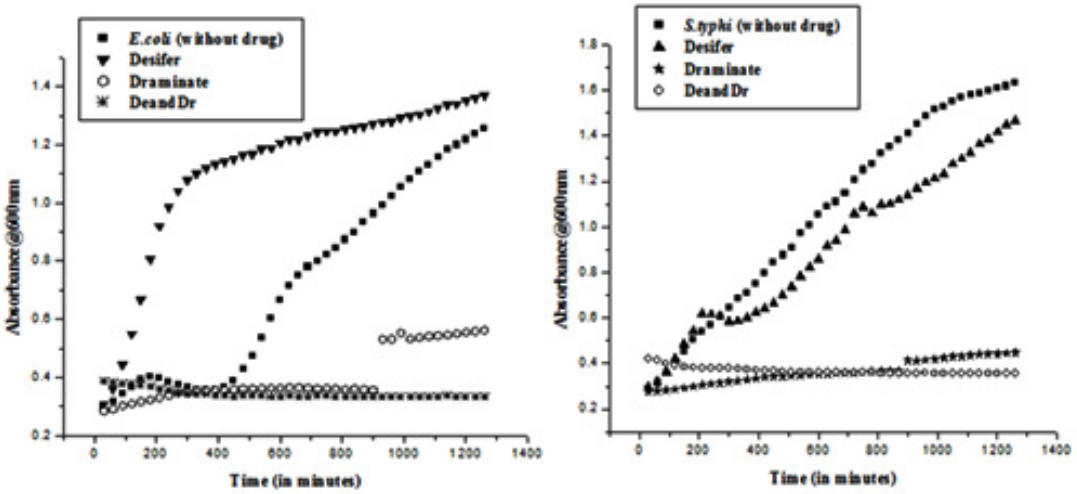

Fig. 4(b). Bactericidal activity of Draminate in combination with Desifer on the growth of *E. coli* and *S. typhi*.

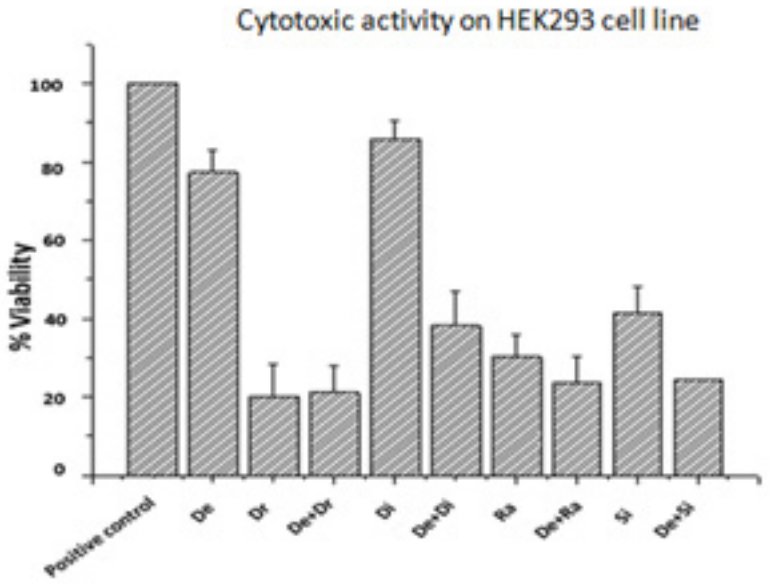

Fig. 5a. Represents the cytotoxic activities of Draminate, Diclofenac, Rantac, and Sinarest alone and in combination with Desifer against the human embryonic kidney (HEK293) cell line

show any antibacterial activity against any of the laboratory cultures.

Determination of the antimicrobial activity of the drug pellet and drug supernatant

The supernatants of all drugs showed antimicrobial activity against at least one laboratory culture. Thus, drug supernatants were used for further experiments.

Determination of the inhibitory effect of different concentrations of Draminate, Diclofenac, Rantac, Sinarest, and Desifer by agar cup diffusion method

Table 2 shows the inhibitory effect of Desifer and the OTC drugs, Draminate, Diclofenac, Rantac, and Sinarest, on laboratory cultures.

The minimum concentrations of selected drugs at which respective microorganisms were inhibited are given in Table 3.No antimicrobial activity was observed against *P. aeruginosa* and *K.pneumoniae*. Further, individual activities of Draminate, Rantac, Sinarest, and Diclofenac and their synergistic activities in combination with Desifer were evaluated.

Determination of the synergistic antibacterial activity of Draminate, Diclofenac, Rantac, and Sinarest in combination with Desifer

Agar cup diffusion method

Table 4 shows the antibacterial effect of Draminate, Diclofenac, Rantac, and Sinarest each in combination with Desifer against laboratory cultures.

Micro broth dilution method

Figure 4 represents the effect of drug combinations on bacterial growth. Figure 4(a) shows that in presence of Desifer, the lag phase was tremendously prolonged till 16hr 40min. At 16hr 40 min., *S. pyogenes* resumes growth as Desifer (desferri form) gets saturated with ferric ions and cannot sequester more ferric ions which get available for the growth of an organism. Table 5 represents the mode of action of drugs against respective laboratory cultures

Analysis of aqueous supernatants of Draminate, Diclofenac, Rantac, Sinarest, and Desifer.

Detection of active components in aqueous supernatant of drugs using thin layer chromatography

Active components in the aqueous supernatant of drugs were determined using thin layer chromatography.

Active pharmaceutical ingredient (API) testing can be done using the most popular non-thermal methods for determining the compatibility of drugs and excipients infrared (IR), near-infrared (NIR), and Raman spectroscopy. Based on their physical and chemical characteristics, these

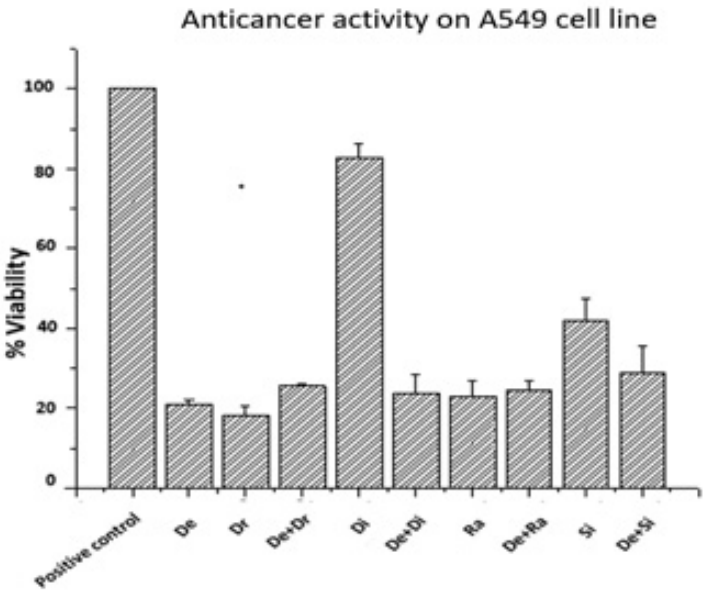

Fig. 5b. Represents anticancer activities of Draminate, Diclofenac, Rantac, and Sinarest alone and in combination with Desifer against human lung cancer cell line A549.

approaches offer a distinctive fingerprint for the API and the excipients

DISCUSSION

To our knowledge, this was the first time that the antimicrobial activity of Draminate, Diclofenac, Rantac, and Sinarest has been assessed in combination with Desifer.

Tablet formulations of OTC drugs were analyzed to determine if these formulations had any antibacterial activities. Rantac, Draminate, and Diclofenac inhibited *M. smegmatis*, *S. typhi, S. aureus, C. diphtheriae*, *S. pyogenes,* and *E. coli,* but were ineffective against *P. aeruginosa* and *K. pneumoniae*.

This indicated that Rantac, Draminate, and Diclofenac had antibacterial activity. This result is as per the fact that all drugs used for treatment possess off-target effects as they share common targets and molecular pathways in cellular functioning.

A similar study had been conducted which supports the findings. In one such study, Draminate was found to have antibacterial activity against some NCTC bacterial cultures,[30] in another study, dimenhydrinate (an active component of Draminate) was found to have antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*.

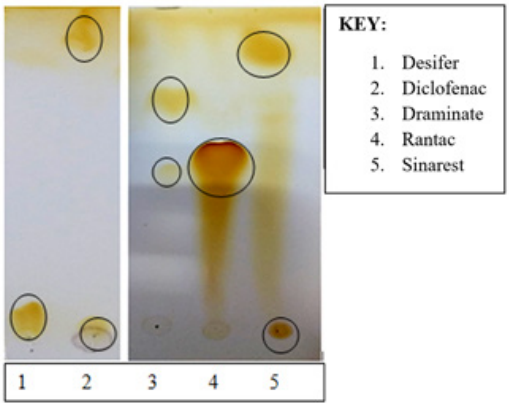

Fig. 6a. Shows the developed thin layer chromatogram loaded with aqueous supernatants of drugs.

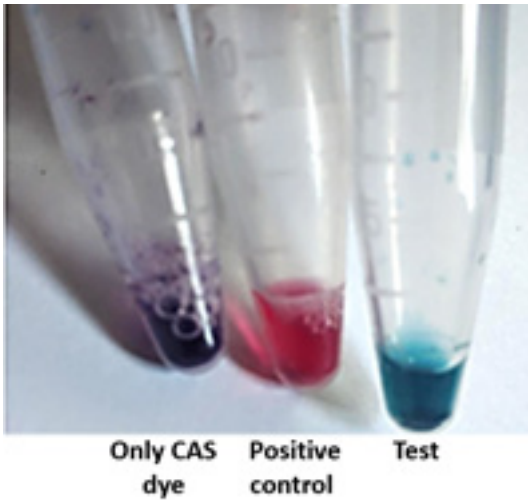

Fig. 6b. Shows the results of the qualitative CAS assay

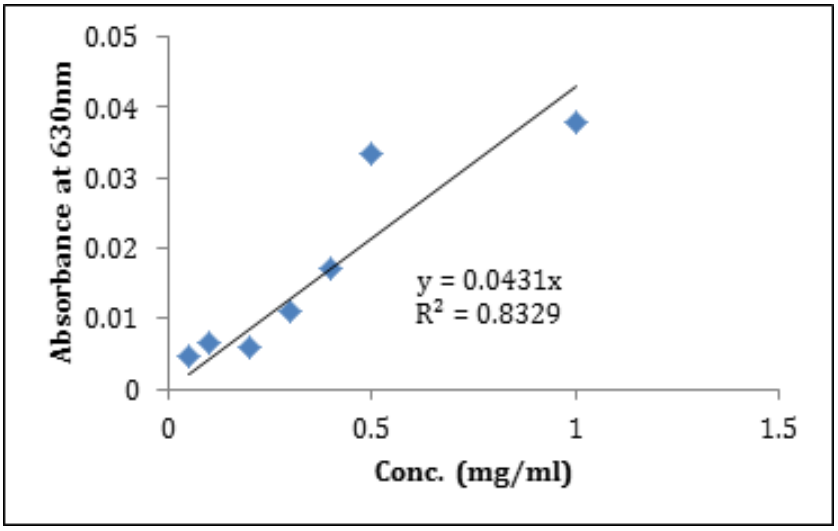

Fig. 6c. Represents the standard graph DFO-B concentrations v/s absorbance at 630nm.

[31] Similarly, Diclofenac, at concentrations of 50-100 µg/ml, was also found to inhibit 397 bacterial strains when tested in-vitro.[32] A study by Sun et al. found two sets of drug combinations that exhibited broad-spectrum antibacterial activity against a panel of ten common MDR clinical isolates. This included *K. pneumoniae, P. aeruginosa, and E. coli,* among others.[33]

The Agar cup diffusion method was used to quantitate the antibacterial activity of the selected drugs in terms of MIC. Based on the results, dilutions were decided to study the inhibitory action of OTC drugs in combination with Desifer using the Agar cup diffusion and Micro broth dilution method. It was found that the OTC drugs had higher MICs when used individually, the MICs decreased to one-fourth of the individual MIC when used in combination with Desifer.

The inhibitory activity of Draminate, Sinarest, Rantac, and Diclofenac was enhanced in the presence of Desifer, this may be due to iron deficiency caused by the sequestration of Fe^{+3}

Table 1. Determination of antimicrobial activities of drug supernatants

Drug Supernatants	Organisms *E.coli*	*S.aureus*	*S.typhi*	*S.pyogenes*	*C.diphtheriae*	*K.pneumoniae*	*M.smegmatis*	*P.aeruginosa*
Rantac	–	–	–	–	±	±	±	±
Diclofenac	+	±	+	±	+	+	+	+
Draminate	±	–	–	–	–	–	–	–
Desifer	+	+	+	+	±	–	–	±
Sinarest	–	±	–	–	–	–	–	–

Key
(+) Clear Zone of Inhibition
(-) No Zone of Inhibition
(±) Partial Inhibition

Table 2. Determination of minimum inhibitory concentration of Desifer and OTC drugs (Draminate, Diclofenac, Rantac, and Sinarest) using agar cup diffusion method

Drug	Conc. (mg/ml)	Zone of inhibition (mm) [size of well=9mm] Organisms *E.coli*	*S.aureus*	*S.typhi*	*S.pyogenes*	*M.smegmatis*	*C.diphtheriae*
Draminate	15	-	-	10	-	-	-
	20	-	-	10	-	-	16*
	25	17*	-	11	-	-	17*
	30	20*	-	11	-	-	-
	35	24*	18.5*	13.5	13	-	-
	40	22*	20*	12	22	-	-
	45	22*	20*	12.5	-	-	-
	50	26*	21.5*	13	20	11	18
Diclofenac	5	-	29*	-	-	2*	-
	10	-	30.5	-	-	24*	-
	15	11*	32	-	-	26*	-
	20	12*	33	-	-	27*	-
Sinarest	250	-	14.5	-	-	-	-
	125	-	13	-	-	-	-
Rantac	25	-	-	12	-	-	-
	50	-	-	13	-	-	-
	75	-	-	13	-	-	-
	100	-	-	16	-	-	-
Desifer	25	-	-	14*	-	-	-
	40	-	19	13*	-	-	15

Key: (-) No inhibition, (*) Partial inhibition

ions. Pathogens have been found to colonize sites in the human body where they have easy access to iron or iron transport systems.[34] This has been observed in pathogens like *Mycobacterium* species, extraintestinal pathogenic *E. coli*, *H. pylori*, and *Burkholderia* species, this shows the essential role of iron in their survival and virulence.[35-38] Iron deficiency causes an increase in cell permeability, thus, the organisms have increased susceptibility to drugs. In addition, iron deficiency may also decrease the growth rate of bacteria. Therefore, the use of Desifer with drugs can be a good strategy to enhance the effectiveness of antibacterial drugs. Nick et al tested Deferoxamine for antimicrobial activity against various human pathogens like *Plasmodium* , *Pseudomonas,* and *Staphylococcus* spp.[39]However, it had high MIC values, and therefore, they studied the activities of Deferoxamine in combination with other antibiotics. This was found to increase antimicrobial activity by 50-fold in some cases.[40] However, in our study, micro broth dilution tests showed different results when Diclofenac was used with Desifer against *S. aureus*. It was found to inhibit *S. aureus* individually, but its inhibitory activity decreased in the presence of Desifer, suggesting that some organisms can survive iron deficiency.

The growth curve analysis was done to identify the bactericidal action of drugs. In combination treatment with Desifer, Sinarest, and Diclofenac showed bacteriostatic activity. The drugs Draminate, Sinarest, and Diclofenac in combination with Desifer showed good inhibitory activity against *M. smegmatis,* suggesting the future use of these Desifer-drug conjugates in the treatment of MDR *M. tuberculosis* infection. Studies have been conducted using artemisinin in combination with Deferasirox against *Mycobacterium tuberculosis* strains, this combination was found effective (Artemisinin alone was found to be ineffective).[41] As liquid

Table 3. MIC of drugs (Draminate, Diclofenac, Rantac, Sinarest, and Desifer) using agar cup diffusion method

Organisms	MIC (mg/ml)				
	Draminate	Diclofenac	Sinarest	Rantac	Desifer
E. coli	>50	>20	-	-	-
S. aureus	>50	10	<125	-	<40
S.typhi	<15	-		<25	>40
S.pyogenes	35	-	-	-	-
M. smegmatis	50	>20	-	-	-
C. diphtheriae	50	-	-	-	40

Table 4. Determination of the antibacterial effect of Draminate, Diclofenac, Rantac, and Sinarest each in combination with Desifer

Drugs (mg/ml)	Zone of inhibition (mm) [size of well=9mm]					
	E. coli	*S.aureus*	*S. typhi*	*S. pyogenes*	*C. diphtheriae*	*M.smegmatis*
Desifer(20)	-	11	-	-	13	11
Draminate(20)	28*	-	-	-	-	-
Desifer(20)+Draminate(20)	30*	15	14	-	13	14
Diclofenac(7.5)	-	-	-	-	-	-
Desifer(20)+Diclofenac(7.5)	-	18	-	-	13*	12
Sinarest(62.5)	-	-	-	-	-	-
Desifer(20)+Sinarest(62.5)	-	14	-	-	12	13
Rantac(25)	-	-	-	-	-	-
Desifer(20)+Rantac(25)	-	12	-	15	-	13

Key: (-) No inhibition (*) Partial inhibition

Table 5. Mode of action of drugs against respective laboratory cultures

Laboratory cultures	Drugs	Activity
E. coli	Draminate(10)	Bacteriostatic
	Desifer (10) + Draminate (10)	Bactericidal
S. typhi	Draminate (10)	Bacteriostatic
	Desifer (10) + Draminate (10)	Bactericidal
S. aureus	Draminate (10)	Bacteriostatic
	Desifer (10) + Draminate (10)	Bactericidal
S. pyogenes	Desifer (10)	Bactericidal
	Desifer (10) + sinarest (31.25)	Bactericidal
	Desifer (10) + Rantac (12.5)	Bacteriostatic
	Draminate (10)	Bacteriostatic
	Desifer (10) + Draminate (10)	Bactericidal
M. smegmatis	Draminate (10)	Bacteriostatic
	Desifer (10) + Draminate (10)	Bacteriostatic
C. diphtheriae	Draminate (10)	Bacteriostatic
	Desifer (10) + Draminate (10)	Bacteriostatic

media is used in the micro broth dilution method, Desifer has better access to ferric ions, leading to increased iron sequestration. Hence, enhanced inhibitory activity was observed using this method compared to the agar cup diffusion method.

These results suggest the use of combination therapy with OTC drugs and Desifer as an alternative therapy. However, the safety of this treatment strategy must be evaluated for its safety, cytotoxicity was checked using HEK293 cell lines. Diclofenac and Desifer exhibited low cytotoxicity, and hence, can be good candidates for repurposing. Some studies have reported diclofenac-induced cytotoxicity in leukocytes due to enzyme-mediated transformation[42], and cytotoxicity in Fibroblast 3T3-L1 preadipocytes[43]. Desifer can also cause cytotoxicity by iron depletion which encourages BclxL downregulation and proximal tubular cell death.[44] However, further investigation is warranted to understand the causes of cytotoxicity.

The OTC drugs like Rantac and Draminate are FDA approved and are consumed commonly by the population taking their safety as guaranteed. However, some studies have shown their toxicity to normal human cell lines. Side effects of OTC medications like antacids, cough, and cold formulation have been investigated where case studies have reported significant morbidity and even mortality in both acute overdoses and when administered in the right dosage but for prolonged periods.[45] Ranitidine-induced anaphylaxis has also been observed.[46]

When checked for anticancer activity against the Human lung cancer cell line A549, drugs Desifer, Draminate, Rantac, and Sinarest individually and in combination with Desifer showed anticancer activity. Although Diclofenac did not show anticancer activity individually, in combination with Desifer, it had anticancer activity, thus, it could be used in combination chemotherapy. Most used drugs exhibit anticancer activity. Rantac has been a part of the Coordinated Undermining of Survival Paths 9 (CusP9) regime for glioblastoma, for which limited therapies are available.[43]Deferasirox demonstrated similar activity in inhibiting the proliferation of DMS-53 lung carcinoma and SK-N-MC neuroepithelioma cell lines.[44] Draminate, Rantac, and Sinarest alone and in combination with Desifer were cytotoxic (fig.5a) as well as had anticancer activity(fig.5b). Cytoxicity may not limit their use, as distinguished anticancer agents usually possess severe side effects at their effective concentrations. However, there are ways to manage the toxicities of anticancer drugs. [45]

TLC analysis was conducted to find several components in the drug supernatants. Desifer and Rantac showed the presence of one component on TLC analysis, Draminate, Sinarest, and Diclofenac showed the presence of two

components each. Chemical testing of Desifer supernatant with CAS dye confirmed the presence of its active ingredient, Deferasirox, furthermore, on TLC analysis, Desifer supernatant showed only one component. Thus, the antibacterial activity of Desifer can be attributed to Deferasirox.

The study has several limitations. All the above experiments were done assuming that the active ingredients of all drugs used were completely water-soluble. Quantitative estimation of deferasirox in Desifer supernatant was done using CAS reagent with the use of DFO-B (injectable) as a standard. It was found that16.7 mg/ml of DFO-B corresponds to 5mg/ml of deferasirox. According to the Merck index, Dimenhydrinate, the active component of Draminate, has a solubility of 3mg/ml in water. However, no analytical methods were used to confirm the concentrations of active ingredients of Draminate, Diclofenac, Rantac, and Sinarest in their aqueous supernatants.

CONCLUSION

Desifer and Diclofenac, Dimenhydrinate, Sinarest, Rantac supernatants individually showed antibacterial activity against laboratory cultures. This inhibitory action may indicate their potential activity against pathogenic strains. The enhanced antibacterial activity of drugs in combination treatment can be attributed to iron deprivation, chelation of essential non-iron metals, and increased membrane permeability due to the action of Desifer. A rifampin and isoniazid-resistant strain of *M. smegmatis* was used as a model for pathogenic MDR *M. tuberculosis* . Commonly used drugs like Draminate, Sinarest, and Diclofenac showed good inhibitory activity against *M. smegmatis* in the presence of Desifer, suggesting the future use of these Desifer-drug conjugates for MDR *M. tuberculosis* infections. However, the safety and efficacy of such a treatment must be analyzed.

In the wake of emerging drug resistance among pathogenic bacteria, such studies would have wide applications in chemotherapy.

ACKNOWLEDGMENT

We would like to thank the Department of Microbiology, St. Xavier's College, Mumbai, for providing us with the laboratory facilities to conduct this work.

Conflict of interest

The author(s) declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Funding Source

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Zou H., Li Z., TianX., Ren, Y. The top 5 causes of death in China from 2000 to 2017. Scientific reports 2022, 12(1), 1-7.
2. Baquero F, Coque TM, Cruz F de la. Ecology and evolution as targets: the need for novel eco-evo drugs and strategies to fight antibiotic resistance. Antimicrobial Agents &Chemotherapy 2011, 8: 3649-3660.
3. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nature Reviews Drug Discovery2006, 6:29–40
4. Tommasi R, Brown DG, Walkup GK, Manchester JI, Miller AA. ESKAPEing the labyrinth of antibacterial discovery. Nature Reviews Drug Discovery 2015, 14:529–542.
5. Baker SJ, Payne DJ, Rappuoli R, De Gregorio E. Technologies to address antimicrobial resistance. Proceedings of the National Academy of Sciences 2018, 115:12887–12895.
6. Oprea TI, Nielsen SK, Ursu O, et al. Associating Drugs, Targets and Clinical Outcomes into an Integrated Network Affords a New Platform for Computer-Aided Drug Repurposing. Molecular Informatics 2018, 30: 100–111.
7. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. Nature Reviews Drug Discovery 2004, 3: 673–683.
8. Chong CR, Sullivan D. New uses for old drugs. Nature 2007, 448: 645–646.
9. Pushpakom S, Iorio F, Eyers P. A., Escott K. J., Hopper S., Wells A., Doig A., Guilliams T., Latimer J., McNamee C., Norris A., Sanseau P., Cavalla D., Pirmohamed M., Drug repurposing: Progress, challenges, and recommendations. Nature Reviews Drug Discovery 2019, **18**, 41–58.
10. Pazhayam NM, Chhibber-Goel J, Sharma A. New leads for drug repurposing against malaria. Drug Discovery Today 2019, (1):263-271.

11. Begley C. G., Ashton M., Baell J., Bettess M., Brown M. P., Carter B., Sullivan M. Drug repurposing: Misconceptions, challenges, and opportunities for academic researchers. Science Translational Medicine 2021, 13(612), eabd5524.
12. D'Amato, R. J., Loughnan, M. S., Flynn, E., & Folkman, J. Thalidomide is an inhibitor of angiogenesis. Proceedings of the National Academy of Sciences 1994, 91(9), 4082-4085.
13. Parman T, Wiley M, Wells P. Free radical-mediated oxidative DNA damage in the mechanism of thalidomide teratogenicity Nature Medicine 1999, 5: 582–585.
14. Wei W, Zhou F, Zhang Y, et al. A combination of thalidomide and arsenic trioxide is effective and well tolerated in patients with myelodysplastic syndromes. Leukemia Research 2012, 36: 715–719.
15. Scherman E, Malak S, Perot C, et al. Interest of the association azacitidine–lenalidomide as frontline therapy in high-risk myelodysplasia or acute myeloid leukemia with complex karyotype. Leukemia 2012, 26: 822–824.
16. Fehniger TA, Byrd JC, Marcucci G, et al. Single agent lenalidomide induces complete remission of acute myeloid leukemia in patients with isolated trisomy 13. Blood 2009, 113: 1002–1005.
17. Ogundeji A, Pohl C, Sebolai O. The repurposing of aspirin and ibuprofen as candidate anti-cryptococcus drugs. Antimicrobial Agents &Chemotherapy 2016, 8: 4799–4808.
18. Gupta SC, Sung B, Prasad S, et al. Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends in Pharmacological Sciences 2013, 34: 508–517.
19. Norman P. Repurposing as a strategy for orphan drug development, evidence from European approvals. Expert Opinion on Orphan Drugs 2013, 1: 473–480.
20. M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Research 2020,* **30**, 269–271.
21. Kifle, Z. D., Ayele, A. G., &Enyew, E. F. Drug repurposing approach, potential drugs, and novel drug targets for COVID-19 treatment. Journal of Environmental and Public Health 2021.
22. Keberle H. The Biochemistry of Desferrioxamine and Its Relation to Iron Metabolism, Ann. N. Y. Acad. Sci 1964, 119: 758–768.
23. Mobarra N, Shanaki M, Ehteram H, Nasiri H, Sahmani M, Saeidi M, Goudarzi M, Pourkarim H, Azad M. A review on iron chelators in treatment of iron overload syndromes. International journal of hematology-oncology and stem cell research. 2016 O10(4):239.
24. Cappellini MD, Cohen A, Piga A, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with â-thalassemia. Blood 2006, 107: 3455–3462.
25. Stumpf JL. Deferasirox. American Journal of Health-System Pharmacy 2007, 64: 606–616.
26. Gokarn K, Pal RB. Preliminary evaluation of anti-tuberculosis potential of siderophores against drug-resistant *Mycobacterium tuberculosis* by mycobacteria growth indicator tube-drug sensitivity test. BMC Complementary and Alternative Medicine 2017, 17: 161.
27. Rice WG, Mercedes Lonergan A. Ditch-Plate Method for Testing Bacterial Resistance to Antibiotics. American Journal of Clinical Pathology 1950, 20: 68–70.
28. Rose SB, Miller RE. Studies with the Agar Cup-Plate Method. Journal of Bacteriology 1939, 38: 525–537.
29. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 1987, 160: 47–56.
30. Dastidar SG, Saha PK, Sanyamat B, et al. Antibacterial Activity of Ambodryl and Benadryl. Journal of Applied Bacteriology 2018, 41: 209–214.
31. Delitheos A, Kefalas S, Kokkinou T, et al. Antibacterial properties of several drug categories. Experientia 1982, 38: 1346–1348.
32. Annadurai S, Basu S, Ray S, et al. Antibacterial activity of the antiinflammatory agent diclofenac sodium. Indian Journal of Experimental Biology 1998, 36: 86–90.
33. Sun W, Weingarten RA, Xu M, Southall N, Dai S, Shinn P, Sanderson PE, Williamson PR, Frank KM, Zheng W. Rapid antimicrobial susceptibility test for identification of new therapeutics and drug combinations against multidrug-resistant bacteria. Emerging microbes & infections 2016, 5:e116.
34. Schaible UE, Kaufmann SHE. Iron and microbial infection. Nature Reviews Microbiology 2004, 2: 946–953.
35. Rodriguez GM, Smith I. Mechanisms of iron regulation in mycobacteria: role in physiology and virulence. Molecular Microbiology 2018, 47: 1485–1494.
36. Gao Q, Wang X, Xu H, et al. Roles of iron acquisition systems in virulence of extraintestinal pathogenic *Escherichia coli*: salmochelin and aerobactin contribute more to virulence than heme in a chicken infection model. BMC Microbiology 2012, 12: 143.

37. Velayudhan J, Hughes NJ, McColm AA, et al. Iron acquisition and virulence in *Helicobacter pylori*: a major role for FeoB, a high-affinity ferrous iron transporter. Molecular Microbiology 2018, 37: 274–286.
38. Butt AT, Thomas MS. Iron Acquisition Mechanisms, and their Role in the Virulence of *Burkholderia* Species. Frontiers in Cellular and Infection Microbiology 2017, 7: 460.
39. Nick H., Acklin P., Lattmann R., Buehlmayer P., Hauffe S., SchuppJ., Alberti D. Development of tridentate iron chelators: from desferrithiocin to ICL670. Current medicinal chemistry 2003, 10(12), 1065-1076.
40. Zurlo M, De Stefano P, Borgna-Pignatti C, Di Palma A, Melevendi C, Piga A, Di Gregorio F, Burattini M, Terzoli S. Survival and causes of death in thalassaemia major. The Lancet 1989, 334(8653):27-30.
41. Miller MJ, Walz AJ, Zhu H, Wu C, Moraski G, Mollmann U, Tristani EM, Crumbliss AL, Ferdig MT, Checkley L, Edwards RL. Design, synthesis, and study of a mycobactin" artemisinin conjugate that has selective and potent activity against tuberculosis and malaria. Journal of the American chemical society 2011, 133(7):2076-9.
42. Monika N, Jiri P, Jan Z, Veronika S. Diclofenac-Induced Cytotoxicity in Cultured Carp Leukocytes. Physiological Research 2020, 69: 607-918
43. Sathishkumar P, Mythili A, Hadibarata T, Jayakumar R, Kanthimathi MS, Palvannan T, Ponraj M, Salim MR, Yusoff AR. Laccase mediated diclofenac transformation and cytotoxicity assessment on mouse fibroblast 3T3-L1 preadipocytes. RSC Advances 2014, 4(23):11689-97.MMartin-Sanchez D, Gallegos-Villalobos A, Fontecha-Barriuso M, Carrasco S, Sanchez-Niño MD, Lopez-Hernandez FJ, Ruiz-Ortega M, Egido J, Ortiz A, Sanz AB. Deferasirox-induced iron depletion promotes BclxL downregulation and death of proximal tubular cells. Scientific reports 2017, 7(1):1-6.
44. Gunn VL, Taha SH, Liebelt EL, Serwint JR. Toxicity of over-the-counter cough and cold medications. Pediatrics 2011, 108(3), e52-e52.
45. Pipkin, G. A, Mills, J. G, Kler L, Dixon J. S. & Wood J. R. The safety of ranitidine bismuth citrate in controlled clinical studies. Pharmacoepidem Drug Safe 1996, 5: 399–407.
46. Gunn VL, Taha SH, Liebelt EL, Serwint JR. Toxicity of over-the-counter cough and cold medications. Pediatrics 2001, 108(3):e52-e52.
47. Purow B. Repurposing existing agents as adjunct therapies for glioblastoma. NeurooncolPract 2016, 3: 154–163.
48. Lui GY, Kovacevic Z, Richardson V, Merlot AM, Kalinowski DS, Richardson DR. Targeting cancer by binding iron: Dissecting cellular signaling pathways. Oncotarget 2015, 6(22):18748.
49. Remesh A. Toxicities of anticancer drugs and its management. International Journal of Basic & Clinical Pharmacology 2017, 1: 2–12.