Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22 (2) : 2020 : 248-253

ISSN-0972-3005

EFFECT OF SILIBININ ON THE SENSITIVITY OF *ENTEROBACTER CLOACAE* RESISTANT ISOLATE TO GENTAMICIN: *IN VITRO* STUDY

WATHIQ MOHAMMED AL-JEWARI[1], ZAID OSAMA IBRAHEEM[2], SAMER SHUKUR MOHAMMED[2], WAEL WALEED MUSTAFA[2] AND SAAD ABDULRAHMAN HUSSAIN[1]*

[1]Department of Basic Sciences, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq

(Received 11 December, 2019; accepted 2 February, 2020)

Key words *: Enterobacter cloacae, Silibinin, Gentamicin, Bacterial resistance*

Abstract – The present study was designed to evaluate the influence of silibinin on the gentamicin activity against the resistant strain of *Enterobacter cloacae*. A gentamicin resistant isolate of *Enterobacter cloaca* was obtained from Al-Sheikh Zayed hospital, Baghdad and cultivated in Mueller Hinton agar; the impact of different concentrations of gentamicin, silibinin or their combination were determined using the conventional drug sensitivity assay to determine the growth inhibitory parameters of each. The study revealed strong resistance for the pathogen against gentamicin and very poor sensitivity for silibinin at the highest concentration used. The isobologram technique was followed to determine the interaction of both drugs against the pathogen. Silibinin antagonizes the gentamicin effect when combined at 7:3, 5:5 and 3:7 ratios. In conclusion, silibinin can augment resistance of *Enterobacter cloacae* to gentamicin when co-administered as a combination. Further studies are recommended to determine the precise mechanism through which gentamicin resistance was conferred and the way through which silibinin antagonized gentamicin.

INTRODUCTION

Enterobacter is a common Gram-negative facultative anaerobic bacterium that causes a number of opportunistic infections in hospitalized immunocompromised patients including lung, urinary tract or peritoneal infections (Mezzatesta *et al*., 2012). Treatment of different Enterobacter-induced infections depends on its resistance profile to different types of antibiotics. The recommended protocol involves four lines of treatment including fourth-generation cephalosporins (cefepime) as the first line, carbapenems as the second line, aminoglycosides especially amikacin and gentamicin as the second line and quinolone derivatives like ciprofloxacin that falls within the fourth line (Thiola *et al*., 2005; Annavajhala *et al*., 2019). The high incidence rate of antibiotic resistance amongst different strains of Enterobacter, as well as prevalence of cross-resistance to more than one antibiotic created a big hassle while prescribing an empirical antibiotic therapy to eradicate the infections induced by these strains (Le-Ha *et al*., 2019; Boutarfi *et al*., 2019). Currently, the availability of effective drugs to eradicate resistant bacteria has been rapidly decreasing, only a few newly approved antibiotics that active against resistant bacteria are clinically used in the past decades (Fernandes, 2015). This urged scientists to search for more potent alternatives or adding chemosensitizers to reverse the antibiotic resistance against conventional antibiotics (Suknasang *et al*., 2019). Silibinin is the major flavonolignan isomer of silymarin isolated from the milk thistle (*Silybum marianum*) seeds (Fibigr *et al*., 2017). This polyphenol is widely used in traditional medicine for the treatment of different liver and gallbladder disorders (de Avelar *et al*., 2017; Boigk *et al*., 1997), in addition to its approval as an antidote for acute cases of *Amanita phalloides* and acetaminophen poisoning (Roberts *et al*., 2013; Campos *et al*., 1988). Many studies have pointed out the antimicrobial effect of different silymarin derivatives and their plausible role in the reversal of antibiotic resistance

in different multidrug-resistant bacteria (de Monbrison *et al*., 2006; Wang *et al*., 2018) including its reversal effect on ampicillin and oxacillin resistance in the methicillin-resistant *Staphylococcus aureus* (MRSA) (de Oliveira *et al*., 2015). Meanwhile, its activity in the reversing effect on chemoresistant cancer cells is well documented (Hussain and Marouf, 2013). As a part of such efforts, the present study aims to evaluate the expected influence of silibinin on the sensitivity of a resistant strain of *Enterobacter cloacae* to the gentamicin effect *in vitro*.

MATERIALS AND METHODS

Materials

Mueller Hinton agar, nutrient broth, and McFarland solution were purchased from Biolab, Hungary. Meanwhile, gentamicin powder was procured from Brawn Laboratories Ltd., India and a standardized powder of silibinin dihemisuccinate (98% purity) was obtained from Tolbiac S.L., Argentina. Samples of *Enterobacter cloaca* resistant strain were isolated from urine samples obtained from patients suffering from urinary tract infection in Al-Sheikh Zayed Hospital, Baghdad. The bacteria were identified and authenticated by a specialized bacteriologist in the Central Public Health Laboratories of the Iraqi Ministry of Health.

Methods

The growth inhibitory effect of gentamicin and silibinin and their combinations were screened using agar diffusion well variant as previously described elsewhere (Valgas *et al*., 2007). The experiment involves the growing and maintenance of the bacteria, exposing the bacteria to different concentrations of the tested compounds and their combination. Inoculums of the bacterial isolates weresuspended in nutrient broth. The bacterial concentration was adjusted at 0.5 McFarland (1-2 $X10^8$ CFU/mL). Then, the bacterial suspension was spread on a sterile Petri dish Mueller Hinton agar using a sterile cotton swab. About 5-6 wells of 7 mm diameter holes and 20 mm apart from each other were cut in the agar gel with the help of a sterile borer before starting the cultivation. Stock solutions containing 10 mM of gentamicin or silibinin were prepared and exposed to a two folds serial dilution to prepare each drug at a concentration range of 1 nM to 1 mM. A constant volume (50 µL) of different concentrations of each compound (1 nM to 1 mM) was added into the wells of the cultivated diffusion agars. The gars were incubated at standard conditions (37 °C and aerobic conditions) for 24 hours. A confluent bacterial growth was observed after inoculation along with the presence of zones of inhibition around the wells. The zones of inhibition for each concentration were measured using a Vernier and utilized for the determination of the growth inhibition parameters (IC_{10}, IC_{50}, IC_{90}, and IC_{99}). Screening of the effects of the silibinin and gentamicin combination was done using the previously described isobolograms technique (Ibraheem *et al*., 2015). Briefly, working solutions of silibinin and gentamicin were prepared from their stocks at concentrations equivalent to 16 times their IC50 against the *Enterobacter cloacae* isolates. The dilution was chosen such that IC50 of each fell in the fourth twofold serial dilution. Then, the two solutions were mixed at fixed ratios (10:0, 7:3, 5:5, 3:7 and 0:10 ratios of gentamicin/silibinin). After that, the mixtures were loaded into the wells made in the cultivation agar at the standard conditions for 24 hours and treated as in drug sensitivity assay to determine the bacterial growth profile and estimate IC_{50} and IC_{90} of each combination separately. For each combination ratio, both FIC_{50} and FIC_{90} (fractional inhibitory concentration) were calculated from the ratio of the drug's IC_{50} or IC_{90} within the combination to those when each compound alone was incubated with the microorganism. An isobologram table was constructed containing both FIC_{50} and FIC_{90} based isobolograms using each of the IC_{50} and IC_{90} data respectively. The interaction is considered synergistic if the FIC value fell below 1, indifference if it was in the range of 1 to 2 and antagonism if the values exceeded the threshold of 2.

RESULTS

The growth inhibitory effect of gentamicin against *Enterobacter cloaca* was demonstrated in Table 1; the results revealed that gentamicin is effective at concentrations quite higher than the acceptable limit to be used in the clinical field since its acceptable plasma level ranges between 5-12 µg/mL. Accordingly, the bacteria are considered as highly resistant to gentamicin. Regarding the bacterial growth inhibitory effects of silibinin, the results showed a slight growth inhibitory effect when it was added at the highest possible concentration (100 mM). Regarding the influence of silibinin on

gentamicin resistance in *Enterobacter cloaca*, it is noteworthy that gentamicin was used at a concentration equivalent to 16 times its IC_{50} against the bacteria (8 mM). Meanwhile, silibinin was used at the highest possible concentration (100 mM) using DMSO as a solvent. The preparation was done such that the DMSO level did not surpass the acceptable threshold against bacterial growth which is 4% v/v. Additionally, it is important to note that a modification was done in the experiment as it was impossible to determine a reliable IC_{50} for silibinin due to its poor activity and inability to rise up the concentration. Its stock was prepared at the highest possible concentration and was used in the combination and the experiment relied on measuring the FIC values of gentamicin only. Surprisingly, an antagonistic effect was revealed when silibinin was combined with gentamicin as the IC_{50} of the later was obviously increased from 0.47 mM when it was given alone to be 3.7, 3.1 and 2.1 mM when both combined at different ratios (7:3, 5:5 and 3:7 gentamicin/silibinin). Furthermore, the IC_{50}-based fractional inhibitory concentrations (FIC_{50}) of the different combinations of gentamicin within the utilized concentrations were higher than 2 (Table 2) indicating a prominent antagonistic effect of silibinin on the antibacterial effect of gentamicin against the resistant strain of *Enterobacter cloacae*. On the other hand, based on the IC_{90}-based fractional inhibitory concentration (FIC_{90}), the antagonistic effect was faded and the interaction was considered as indifference (Table 2).

Table 1. Growth inhibitory effect of gentamicin against *Enterobacter cloacae* resistant isolate

Concentration Unit	Parameters			
	IC_{10}	IC_{50}	IC_{90}	IC_{99}
mM	0.0277	0.47	8	15.1
mg/mL	13.3	225	3830	7249

DISCUSSION

Silibinin is the major flavonolignan derivative of silymarin that widely used in traditional medicine as a tonic agent for liver and gallbladder. Many studies clearly demonstrated its various biological effects including anticancer, anti-fibrotic, antiangiogenic and antioxidant effects (Jahanafrooz *et al*., 2018; Pivodová *et al*., 2016; Ou *et al*., 2018). Moreover, the antimicrobial activity of silibinin was highlighted by many investigators against different pathogens (de Oliveira *et al*., 2015; Yun and Lee, 2017). Furthermore, silibinin has been reported to reverse antibiotic resistance in several types of bacterial strains, especially its synergy with ampicillin and oxacillin against MRSA (Kang *et al*., 2011). This feature encouraged evaluation of its use as a chemosensitizer for gentamicin against the resistant strain of *Enterobacter cloacae*. Enterobacter is one of the opportunistic pathogens that trigger serious infections in immunocompromised patients (Arias *et al*., 2010). Dissemination of antibiotic resistance amongst different strains of Enterobacter has perplexed the dedicated efforts to eradicate them (Liu *et al*., 2015); this encourages the search for alternatives or new chemosensitizers that can enhance the antimicrobial effects of antibiotics on resistant microbial strains. The clinical isolate used in the present study was highly resistant to gentamicin (Table 1). Aminoglycosides resistance is highly disseminated worldwide and led to the loss of its token as an effective antibiotic to treat different infections. This incidence adds more burdens to health authorities as it turns the cost-effective aminoglycoside derivatives useless to eradicate different infections (Krause *et al*., 2016). It has been suggested that resistance to the aminoglycosides develops via four main mechanisms, including enzymatic modification and inactivation via acetylation using aminoglycosides acetyltransferase enzyme, nucleotides conjugation via nucleotides-transferase enzyme or phosphorylation via phosphotransferase enzyme. These mechanisms are highly plausible in Enterobacter, as previous reports dictated its higher incidence in the gram-negative bacteria rather than in the gram-positive type (Garneau-Tsodikova and Labby, 2016). On the other

Table 2. Drugs combination assay (isobologram technique) for gentamicin and silibinin at different ratios

Gentamicin/ Silibinin	Gentamicin IC_{50}mM	Gentamicin FIC_{50} mM	Gentamicin IC_{90} mM	Gentamicin FIC_{90} mM
1:0	0.47	1.00	8.0	1.0
7:3	3.7	7.54	14.5	1.74
5:5	3.09	6.13	12.5	1.53
3:7	2.1	4.15	8.3	1.16

hand, the resistance may be conferred also via stimulation of aminoglycosides efflux by the multidrug resistance mechanism or a decrease in the permeability of bacterial cell wall to the drug. Aminoglycosides cross the cell wall through binding to external cations (Mg^{+2} and Ca^{+2}) that link the cell wall lipopolysaccharides together. This binding results in the further partial destruction of the membrane and the influx of more molecules into the bacteria (Garneau-Tsodikova and Labby, 2016). Different bacteria have different strategies for the induction of aminoglycosides resistance; however, molecular modification of the aminoglycosides or alterations in the target sites is the most prevalent mechanism (Szymanek-Majchrzak *et al*., 2018). In the present study, the utilized resistant bacterial is not merely poorly sensitive to gentamicin; it showed a very poor sensitivity to silibinin indicating that this polyphenol is unsuitable to be implemented as an antibacterial agent. However, based on previously reported data (Cai *et al*., 2018), one may expect its ability to change the antimicrobial effect of the gentamicin. Surprisingly, the antagonistic effect was obtained when the isolated *Enterobacter cloacae* were exposed to different mixtures of silibinin and gentamicin. The extent of antagonism was higher when they were combined at a higher ratio of gentamicin. This interaction was recognized more in the FIC_{50}-based isobologram rather than the FIC_{90}. This indicates that it may show a higher resistance augmenting effect than the induction of drug tolerance. In chemotherapy, the term resistance is mostly related to the dose required to eradicate the pathogen and correlated with IC_{50}; meanwhile, tolerance is related to the protraction of the required exposure period to kill the pathogen (Michiels *et al*., 2016). The results of the present study showed controversy with other studies that reported ubiquity of a synergistic effect of silibinin when added with penicillin derivatives (Lee *et al*., 2012). This controversy is possible due to the plenitude of the mechanisms through which different bacteria develop resistance to different antibiotics and the diversity in the mechanisms through which the antibiotics produce their effects. Penicillins inhibit the building of the peptidoglycan matrix in the bacterial cell wall via binding to a specific penicillin-binding protein (PBPs). Alteration in the PBPs is one of the main mechanisms to confer resistance. On the other hand, it may be conferred by the expression of a β-lactamase enzyme that breaks down the β-lactam ring of different penicillin derivatives (Yocum *et al*., 1979). Synergy with penicillin derivatives may be conferred through enhancement their binding to the target site or through inhibition of the â-lactamase which is the most plausible mechanism (He *et al*., 2016). Accordingly, it is clear that the story with aminoglycosides is different and this difference may have imparted in the creation of the discrepancy in the results of the present study. Overall, in this occasion, it is suggested that silibinin may antagonize gentamicin through different pathways including inhibition of its permeation into the intracellular compartment, triggering efflux of gentamicin outside the cell, enhancement of gentamicin modification or induction of gentamicin binding to its target site of the ribosome via competitive or non-competitive binding. This requires further molecular studies to confirm the exact mechanism through which the antagonism was conferred. One study has suggested the effect of silibinin on the bacterial cell wall, which can be attributed to its lipophilicity that triggers its accumulation in the lipid bilayer (Radhika *et al*., 2017). Its accumulation in the membrane might interfere with the permeation of gentamicin into the cell and augmented the resistance. Phytochemicals are pluripotential molecules with different intracellular functions (Upadhyay and Dixit, 2015). It is plausible also that silibinin might have enhanced efflux of gentamicin outside the cell through augmentation of the multidrug resistance mechanism.

CONCLUSION

The present study suggests that caution should be exercised while taking different phytochemicals with antibiotics as their combination does not always lead to a synergistic effect. Antagonism is expected as antibiotic resistance is developed by different mechanisms for different antibiotics and in different bacteria.

ACKNOWLEDGMENT

The authors gratefully thank Al-Rafidain University College for supporting the project and Al-Sheikh Zayed Hospital for technical support.

REFERENCES

Annavajhala, M.K., Gomez-Simmonds, A. and Uhlemann, A.C. 2019. Multidrug-resistant *Enterobacter cloacae*

complex emerging as a global, diversifying threat. *Front. Microbiol*. 10 : 44.

Arias, C.A. and Contreras, G.A. and Murray, B.E. 2010. Management of multidrug-resistant enterococcal infections. *Clin. Microbiol. Infect*. 16 (6) : 555-562.

Boigk, G., Stroedter, L., Herbst, H., Waldschmidt, J., Riecken, E.O. and Schuppan, D. 1997. Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. *Hepatology*. 26 (3) : 643-649.

Boutarfi, Z., Rebiahi, S.A., Morghad, T., Pulido, R.P., Burgos, M.J.G., Mahdi, F., Lucas, R. and Galvez, A. 2019. Biocide tolerance and antibiotic resistance of *Enterobacter* spp. isolated from the Algerian hospital environment. *J. Glob. Antimicrob. Resist*. Pii: S2213-7165(19) : 30094-3.

Cai, J.Y., Li, J., Hou, Y.N., Ma, K., Yao, G.D., Liu, W.W., Hayashi, T., Itoh, K., Tashiro, S.I., Onodera, S. and Ikejima, T. 2018. Concentration-dependent dual effects of silibinin on kanamycin-induced cell death in *Staphylococcus aureus*. *Biomed. Pharmacother*. 102 : 782-791.

Campos, R., Garrido, A., Guerra, R. and Valenzuela, A. 1988. Acetaminophen hepatotoxicity in rats is attenuated by silybin dihemisuccinate. *Prog. Clin. Biol. Res*. 280 : 375-378.

de Avelar, C.R., Pereira, E.M., de Farias-Costa, P.R., de Jesus, R.P. and de Oliveira, L.P.M. 2017. Effect of silymarin on biochemical indicators in patients with liver disease: Systematic review with meta-analysis. *World J. Gastroenterol*. 23 (27) : 5004-5017.

de Monbrison, F., Maitrejean, M., Latour, C., Bugnazet, F., Peyron, F., Barron, D. and Picot, S. 2006. In vitro antimalarial activity of flavonoid derivatives dehydrosilybin and 8- (1;1)-DMA-kaempferide. *Acta Trop*. 97 (1) : 102-107.

de Oliveira, D.R., Tintino, S.R., Braga, M.F., Boligon, A.A., Athayde, M.L., Coutinho, H.D., de Menezes, I.R. and Fachinetto, R. 2015. *In vitro* antimicrobial and modulatory activity of the natural products silymarin and silibinin. *Biomed Res. Int*. 2015 : 292797.

Fernandes, P. 2015. The global challenge of new classes of antibacterial agents: an industry perspective. *Curr. Opin. Pharmacol*. 24 : 7-11.

Fibigr, J., Šatínský, D. and Solich, P. 2017. A new approach to the rapid separation of isomeric compounds in a *Silybum marianum* extract using UHPLC core-shell column with F5 stationary phase. *J. Pharm. Biomed. Anal*. 134 : 203-213.

Garneau-Tsodikova, S. and Labby, K.J. 2016, Mechanisms of resistance to aminoglycoside antibiotics: Overview and perspectives. *Med. Chem. Comm*. 7 (1) : 11-27.

He, M., Shao, L., Liu, Q., Li, J., Lin, H., Jing, L., Li, M. and Chen, D. 2016. Mechanism of synergy between SIPI-8294 and â-lactam antibiotics against methicillin-resistant *Staphylococcus aureus*. *Lett. Appl. Microbiol*. 63 (1) : 3-10.

Hussain, S.A. and Marouf, B.H. 2013. Silibinin improves the cytotoxicity of methotrexate in chemoresistant human rhabdomyosarcoma cell lines. *Saudi Med. J*. 34 (11) : 1145-1150.

Ibraheem, Z.O., Abdul Majid, R., Mohd Noor, S., MohdSidek, H. and Basir, R. 2015. The Potential of â carboline alkaloids to hinder growth and reverse chloroquine resistance in *Plasmodium falciparum*. *Iran J. Parasitol*. 10 (4) : 577-583.

Jahanafrooz, Z., Motamed, N., Rinner, B., Mokhtarzadeh, A. and Baradaran, B. 2018. Silibinin to improve cancer therapeutic, as an apoptotic inducer, autophagy modulator, cell cycle inhibitor, and microRNAs regulator. *Life Sci*. 213 : 236-247.

Kang, H.K., Kim, H.Y. and Cha, J.D. 2011. Synergistic effects between silibinin and antibiotics on methicillin-resistant *Staphylococcus aureus* isolated from clinical specimens. *Biotechnol. J*. 6(11) : 1397-1408.

Krause, K.M., Serio, A.W., Kane, T.R. and Connolly, L.E. 2016. Aminoglycosides: An Overview. *Cold Spring Harb. Perspect. Med*. 6(6) : pii:e027029.

Lee, Y.S., Jang, K.A. and Cha, J.D. 2012. Synergistic antibacterial effect between silibinin and antibiotics in oral bacteria. *J. Biomed. Biotechnol*. 2012 : 618081.

Le-Ha, T.D., Le, L., Le-Vo, H.N., Anda, M., Motooka, D., Nakamura, S., Tran, L.K., Tran, P.T., Iida, T. and Cao, V. 2019. Characterization of carbapenem- and colistin-resistant *Enterobacter cloacae* carrying Tn6901 in bla NDM-1 genomic context. *Infect. Drug Resist*. 12: 733-739.

Liu, J., Zeng, T., Su, G., Lin, L.Y., Zhao, Y., Yang, W.Q., Xie, W.X., Zhao, Z.G. and Li, G.M. 2015. The dissemination mode of drug-resistant genes in *Enterobacter cloacae*. *Indian J. Med. Microbiol*. 33: 87-92.

Mezzatesta, M.L., Gona, F. and Stefani, S. 2012. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. *Future Microbiol*. 7 : 887-882.

Michiels, J.E., Van den Bergh, B., Verstraeten, N. and Michiels, J. 2016. Molecular mechanisms and clinical implications of bacterial persistence. *Drug Resist. Updat*. 29 : 76-89.

Ou, Q., Weng, Y., Wang, S., Zhao, Y., Zhang, F., Zhou, J. and Wu, X. 2018. Silybin alleviates hepatic steatosis and fibrosis in NASH mice by inhibiting oxidative stress and involvement with the Nf-êB pathway. *Dig. Dis. Sci*. 63 (12) : 3398-3408.

Pivodová, V., Zahler, S., Karas, D., Valentová, K. and Ulrichova, J. 2016. *In vitro* study of 2,3-dehydrosilybin and its galloyl esters as potential inhibitors of angiogenesis. *Pharmazie*. 71 (8) : 478-483.

Radhika, M.I., Ezhilarasan, D. and Gopinath, P. 2017. Antimicrobial efficacy of silymarin and silibinin against oral microorganisms. *J. Microbiol. Infect. Dis*. 7 (3) : 139-143.

Roberts, D.M., Hall, M.J., Falkland, M.M., Strasser, S.I. and Buckley, N.A. 2013. *Amanita phalloides* poisoning and treatment with silibinin in the Australian Capital Territory and New South Wales. *Med. J. Aust*. 198 (1): 43-47.

Suknasang, S., Teethaisong, Y., Kabkhunthod, S., Mingsiritom, N., Chueakwon, P. and Eumkeb, G. 2019. Antibacterial activity of colistin is resurrected by *Stephaniasuberosa* Forman extract against colistin-resistant *Enterobacter cloacae*. *Lett. Appl. Microbiol*. 69 (2) : 128-135.

Szymanek-Majchrzak, K., Mlynarczyk, A., Kawecki, D., Pacholczyk, M., Durlik, M., Deborska-Materkowska, D., Paczek, L. and Mlynarczyk, G. 2018. Resistance to aminoglycosides of methicillin-resistant strains of *Staphylococcus aureus*, originating in the surgical and transplantation wards of the Warsaw clinical center-A retrospective analysis. *Transplant Proc.* 50 (7) : 2170-2175.

Thiolas, A., Bollet, C., La Scola, B., Raoult, D. and Pagès, J.M. 2005. Successive emergence of *Enterobacter aerogenes* strains resistant to imipenem and colistin in a patient. *Antimicrob. Agents Chemother*. 49 (4) : 1354-1358.

Upadhyay, S. and Dixit, M. 2015. Role of polyphenols and other phytochemicals on molecular signaling. *Oxid. Med. Cell. Longev*. 2015 : 504253.

Valgas, C., de Souza, S.M., Smânia, E.F.A. and Smânia, A. 2007. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol*. 38 : 369-380.

Wang, D., Xie, K., Zou, D., Meng, M. and Xie, M. 2018; Inhibitory effects of silybin on the efflux pump of methicillinresistant *Staphylococcus aureus*. *Mol. Med. Rep*. 18 (1) : 827-833.

Yocum, R.R., Waxman, D.J., Rasmussen, J.R. and Strominger, J.L. 1979. Mechanism of penicillin action: penicillin and substrate bind covalently to the same active site serine in two bacterial D-alanine carboxypeptidases. *Proc. Natl. Acad. Sci. USA*. 76 (6): 2730-2734.

Yun, D.G. and Lee, D.G. 2017. Assessment of silibinin as a potential antifungal agent and investigation of its mechanism of action. *IUBMB Life*. 69 (8) : 631-637.