The effect of Silver Bio-Nanoparticles Synthesized by *Curcuma longa* L. on pathogenic fungi

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Abstract: Silver has reported as inhibitor agent on medical and industrial fungi .The first light implementation of silver and silver nanoparticles(AgNPs) involves in medical industrialization such as ointments to get rid of the infection of open wounds and burns, over and above these biologically synthesized nanoparticles are highly toxic against human pathogens that showed resistance to different medications. This research encompassed the test of *Curcuma longa* L extract and Biosynthesis of silver nanoparticles by *Curcuma longa* L against four pathogenic fungi *Aspergillus niger* ,*P. nigricans, P. iticalum* and *Penicillum sp*, Curcuma longa extract has no influence on *A.niger* growth rate in comparison with the other three fungi where the radial growth rate averged (7.6, 8.3 , 2.8)cm for the three fungi abovementioned sequentially .The synthesis of silver nanoparticles examined by the alteration of color (color changed to yellow) and for the confirmation of AgNPs synthesis,UV-Vis spectroscopy was exploited for this destination at absorption between (291.5-663.5) nm and its antifungal action was evaluated .AgNPs has been the greatest inhibition ability,where the growth rate of penicillum .sp was tremendously inhibited (0.4)cm . The uttermost radial growth of *P.nigricans* and *P.iticalum* reached (6. 3 , 5. 4)cm,while *A.niger* was less susceptible compared to control.The result of this study proves that silver nanoparticles may be serve as effective inhibition factors versus pathogenic fungi.

Keywords: Silver Nanoparticles , Antifungal activity ,*Curcuma longa L.*,Pathogenic fungi

Introduction

Now days ,an intensive investigation has been performed to create new medications from natural products due to the resistance of micro-organisms to present drugs. Nature served as a significant source for the products that presently being used in the improvement of medical practice[1] and to chieve that ,Many techniques were followed by scientists, but the most significant techniques that involved in recent century is Nanotechnology ,where Nanotechniques were applied in technology solutions and has widespread applicative aspects in different research areas such as medicine, computational sciences Biology ,chemistry and physics [2,3, 4, 5] .The use of plants and microbes in the synthesis of nanoparticles has been currently used for several reasons .The first reason is that they environmentally and biologically safe and may be serve as innocuous

nan- factories ,another reasons is they a lower cost and safe for human therapeutic use [12, 13]. It quite important to point out that many microorganisms is hygienically risk to all living creatures, and care must be taken in to account for the production of nanoparticles. It is shown that certain plants have ability to form bio-nanoparticles that act as toxic materials such as metallic ions [5]. In the biosynthesis of metal nanoparticles by plant or plant extract, Silver has long been recognized as an effective antimicrobial agent that exhibits low toxicity to humans and has diverse in vitro and in vivo applications [7] Recently, silver-based topical dressings are widely used to treat infections in open wounds and chronic ulcers as antimicrobial agents. These dressings also protect the host material from oxidation and discoloration [8, 9]. Nanoparticles biologically constituted by plant extract proffer various benefits over chemical and physical levels [10,11,12, 13]. Plant extracts was exploited by many scientists to synthesize nanoparticles, particularly Lantana camara [11], Moringa oleifera (14), Catharanthus roseus [15] and Eucalyptus chapmaniana [16]. *Curcuma longa* has various medical charachteristics Linn. Rhizome of Haridra is widely known to have therapeutic actions and practitioners used it as an anti-diabetic [8,10] hypolipidemic [8,11] anti-inflammatory [10,11] anti-diarrhoeal [9] hepatoprotective [8,9].Anti-asthmatic and anti-cancerous drug.Haridra is considerably used in cosmetology [2]. Latent of the plants as bio-resources materials synthesize Nano-metals is still under investigation. This is the first endeavor to assess the antifungal effectiveness of silver nanoparticles synthesized by *Curcuma longa* L on four pathogenic fungi

Methodology

Materials

The chemical silver nitrate (AgNO3) , *Curcuma longa* L., Distilled water, Potato's Dextrose Agar (PDA) .

Preparation of the filtrate of the *Curcuma longa* L.

The preparation of Curcuma longa extract was made by addition 10gm of Curcuma longa powder to 100ml distilled water and incubated for 7 days a t room temperature, then the extract were filtered with the help of filter paper , after that extract was kept in refrigerator at 4 C for future experiments.

Synthesis of AgNPs

Aqueous solution of silver nitrate was prepared by adding 2mM of AgNO3 to 90 ml of distilled water at room temperature. The aqueous solution was mixed with 10ml of Curcuma longa L. at 70 °C, while stirring magnetically at 1000 rpm for 10 min. The UV–Vis spectroscopy characterization was achieved by using the bio-reduced aqueous component

Characterization of AgNPs

UV-Vis spectrophotometer (CE7200) was involved to confirm the characterizing of AgNPs at wavelength between200-900 nm.

Evaluation of antifungal activity

The silver nanoparticles bio- formed by Curcuma longa L. was tested for assaying the antifungal activity by Poisoned food method [17] versus different pathogenic fungi *Aspergillus niger, penicillum sp , p. italicum and p. nigricans*.The pure cultures of fungi were sub cultured on PDA. Disk of each fungi was transferred from the colony of fungi using Piercing cork diameter 7.5 on to each well on all plates . The radial growth of fungi was measured after incubation for 7 days at 27◦C.

Results and Discussion

Curcuma longa proved its ability to form silver nanoparticles and change in color is a decisive clue for the formation of Nanoparticles in aqueous solution [18] , Colour alteration take place because of the excitation of surface Plasmon resonance phenomenon. [19] pointed out that the conversion of the original solution color through the test of nanoparticles formation by medicinal plant extracts refers to the synthesis of silver nanoparticles (AgNPs) . The results of this investigation explained that the color of aqueous solution changed from dark brown to yellow after the addition of silver nitrates and heating for 10 minutes compared to control as show in **Figure(1)**.

It can be observed that the previous yellow color of

the reaction mixture is changed to the yellowish color after 10 min of reaction. The emersion of yellow color in solution is a proof for the formation of silver nanoparticles in the interaction admixture. The excitation of surface plasmon vibrations is the cause of solution coloring particularly the group conduction electron oscillation in the AgNPs[20]. Vis spectroscopy is a very important and practical technique in the nanoparticles analysis was exploited for characterization of nanoparticles biologically synthesized by plant extracts Optical spectroscopy is vastly exploited for the characterization of nanomaterials where used to confirm the formation of AgNPs which showed a strong peak at 663.5 nm **figure(2).** The strong absorption peak at long wave lengths refers to the less organic compounds widely known known to react with silver ions. The band of surface plasmon in the AgNPs solution remains about 380 nm during the period of reaction pointing out that particles are disbanded in the aqueous solution and no proof for accumulations[21].

In plants the probability of reduction of AgNO3 to silver can be illustrated seeing that the mechanism said to be glycolysis,which involves CO2 fixation with availability of sunlight, where Carbohydrates formed [22].

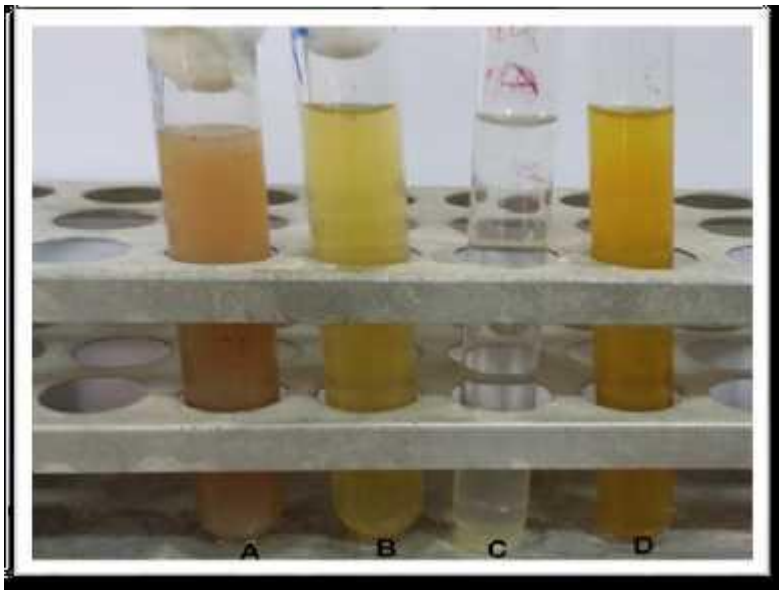

Figure 1 : A : The *Curcuma longa* L. after immersion in AgNO3 solution for 10 min after heating
B : The *Curcuma longa* L. after immersion in AgNO3 solution without heating
C: The tube containing only AgNO3 solution
D: The tube containing only *Curcuma longa* L extract

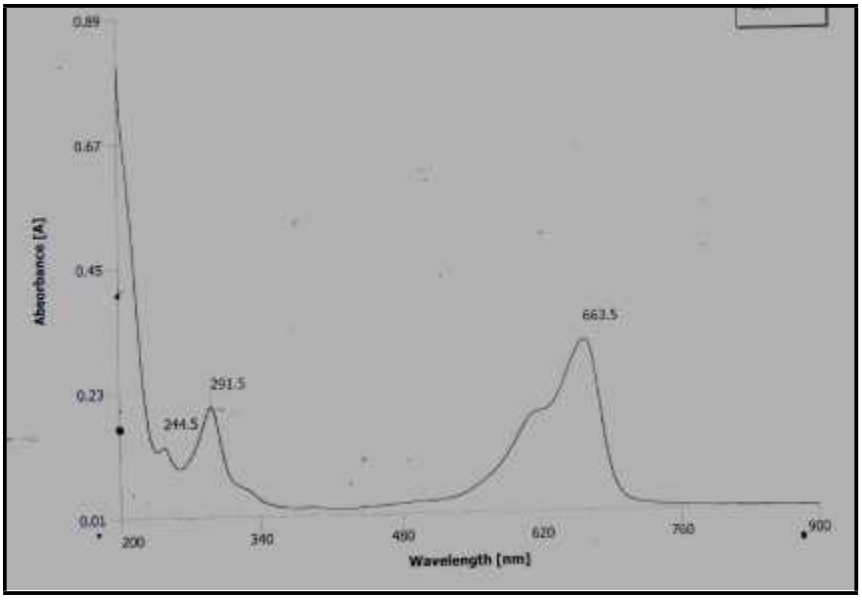

Figure (2) UV/Vis absorption spectra of reduction of silver ions to silver nanoparticles after heating at 70 °C for 10 min

The applications of nanotechnology in medicine can open doors for best investigations on the toxicity of pathogens. Biological synthesis of Nano particles by natural products especially plant extracts has attracted the attention of many scientists to control diseases. In this study, the impact of AgNPs on pathogenic fungi was investigated. The zone of inhibition increased according to concentration of AgNPs **Figure (4).** AgNPs proved their ability to inhibit the growth of Pathogenic fungi as illustrated in **figure(3).** where the radial growth of *A.niger* amounted (7.9)cm . Curcuma longa L extract has no effect on the growth of fungus on PDA, The growth rate was (7.6, 8.3 , 2.8)cm of *P.nigricans P.iticalum* and *Penicillum sp* respectively compared to control when they treated only with the extract of *Curcuma longa* L. Bionanoparticles have the most important role in the inhibition of the growing rate of the abovementioned three fungi ,the radial growth amounted to (6.3, 5.4, 0.4)cm sequentially in comparison with their natural growth on PDA.

Plant extracts have a potent influence on diverse microorganism including animal,human and plant pathogens and the effectiveness of silver as antifungal agents belongs to the virotoxicity[23]. The strong interference of silver ions with the thiol group of vital enzymes and obstruct the enzyme actions[24]. Another reason is that the replication of DNA is obstructed as soon as the micro-organism exposed to ionic silvers[23,16] the affection of nanoparticles can lead to the denaturation the structure of the pathogen proteins resulting in atrophy due to the stabilization ofnanoparticle as a colloid in the medium The NPs biologically formed by plant species have high toxicity on drug resistant microorganisms because they have great potential in the biomedicine research areas, Such as the investigations that achieved on *Allium cepa*[25],*Argimone Mexicana*[26] *Artocarpus heterophyllus*[27]. The interaction of silver nanoparticles with metabolic pathway of pathogens has been well studied[28]. The infiltration of metallic ions across the microsomal membrane through a biological process brought about to the inhibition of oxidation[29]

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The enzymes which reduce a salt to its metallic solid nanoparticles through the catalytic effect are released by the bio-formation of metal nanoparticle [17].The synthesis of silver nanoparticles proffer a number of benefits of eco-friendliness and compatibility in medical and biological applicative uses as they do not use toxic chemicals for the synthesis protocol, for this reason ,they used as a disinfectant; such as, the in medication of injuries and burns seeing that of its toxicity to bacteria AgNPs have unique catalytic, optical, electrical and antimicrobial properties[18]

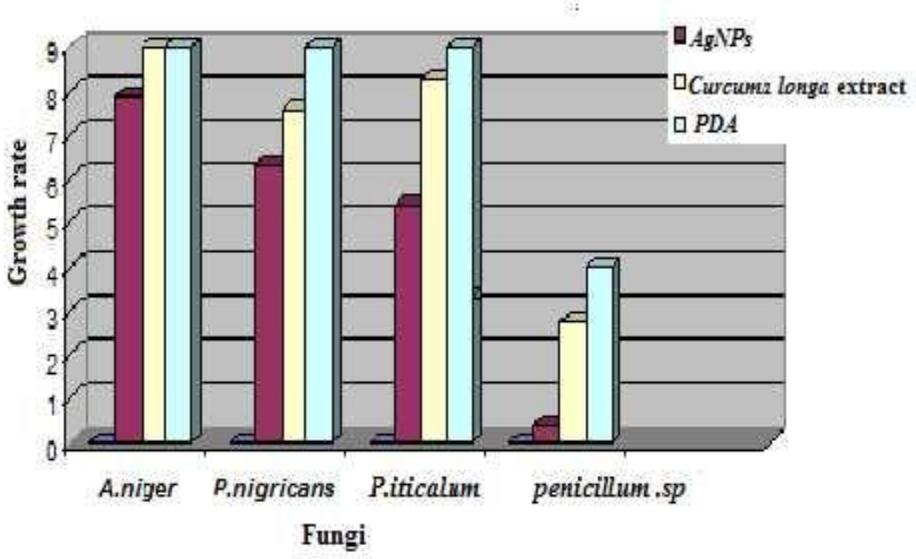

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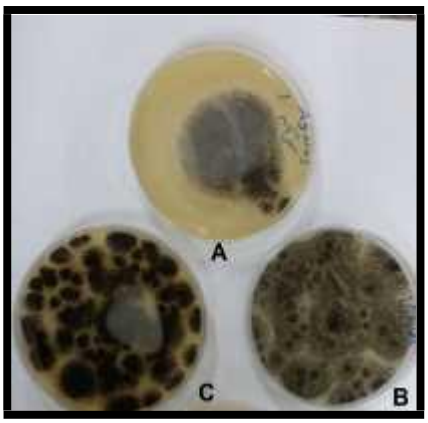
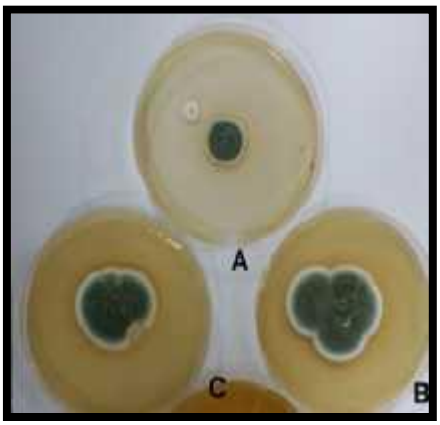
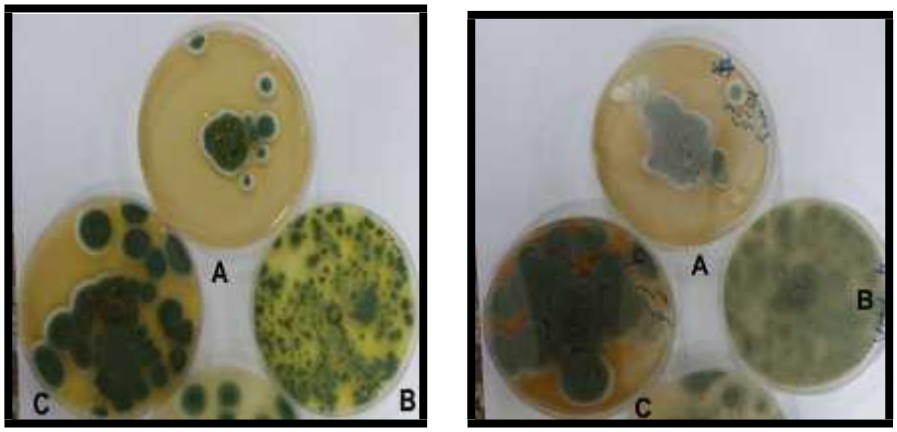
Figure 3 : aqueous AgNPs with *Curcuma longa* L treatment had significant inhibited effect on the growth of tested fungi



(1) *Aspergillus niger*



(2*) P. nigricans*



(3*) P. iticalum* **(4) *Penicillum sp*.**

Figure 4 :**A** –Inhibition zone made by AgNPs **B**-The growth of fungi on PDA **C**—Inhibition zone made by *Curcuma longa* L extract

Conclusion

In the current paper, the formation of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by curcuma longa powder extract was reported for the first time UV-visible spectrophotometer was used to record optical absorption spectrum of silver Nano particles as well as its affectivity versus different pathogenic fungi was assayed, where the growth of fungi was highly suppressed by AgNPs and this suggests that they have a potent efficiency to prepare medicines .

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References:

1- Linton JD, Walsh S, Integrating innovation and learning curve theory: An enabler for moving nanotechnologies and other emerging process technologies into production. R&D Management, 2004. 34: 513-522.

2- Kautt M, Walsh S, Bittner K, Global distribution of micro-nano technology and fabrication centers: A portfolio analysis approach. Technology Forecasting and Social Change , 2007,74:1697-1717.

3- Freitas Jr RA,The Future of Nanomedicine. Futurist , 2010,44(1): 21-22

4- Hyungsub C, Mody CCM, The Long History of Molecular Electronics: Microelectronics Origins of Nanotechnology. Social Studies of Science (Sage) , 2009,39(1): 11-50.

5- Mehra RK, Winge DR , Metal Ion Resistance in Fungi: Molecular Mechanisms and their Regulated Expression. J. Cell. Biochem, 1991, 45 30-40

6- Farooqui, M.D.A.; Chauhan, P.S.; Krishnamoorthy, P.; Shaik, J,Extraction of silver Nanoparticle from the leaf extracts of Clerodendrum

inerme,Dig.J.Nanomat.Biostruct, 2010,543-549.

7- Upendra, K.P.; Preeti, S.S.; Anchal, S, Bioinspired synthesis of silver nanoparticles. Dig. J. Nanomater. Biostruct, 2009, 4 :159‑ 166 .

8- Dipankar, C.; Murugan, S,The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. Colloids and Surfaces B: Biointerfaces., 2012, 98: 112-119.

9- Sunkar, S.; Nachiyarm CV,Microbial synthesis and characterization of silver nanoparticles using the endophytic bacterium Bacillus Cereus: A Novel Source in the Benign Synthesis. Global J Med Res, 2012, 12(2): 43-50.

10- Schneidewind, H.; Schuler, T.; Strelau, K.K.; Weberm K.; Cialla,D.; Diegel, M,The morphology of silver nanoparticles prepared by enzyme-induced reduction. Beilstein J Nanotechnol, 2012, 3: 404-414.

11- Sivakumar, P.; Nethra Devi, C.; Renganathan, S, Synthesis of silver nano particles using Lantana camara fruit extract and its effect on pathogens. Asian J Pharm Clin Res, 2012, 5(3): 97-101

12- Kouvaris, P.; Delimitis ,A.; Zaspalis, V.; Papadopoulos, D.; Tsipas,SA.; Michailidis, N,Green synthesis and characterization of silvernanoparticles produced using Arbutus Unedo leaf extract. Materials Lett, 2012,76: 18-20

13- Kumar, V.; Yadav, S. K., Plant-mediated synthesis of silver and gold nanoparticles and their applications. J. Chem. Technol, 2009, 84: 151-157

14- Prasad, T.N.V.K.V.; Elumalai, EK, Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity. Asian Pac J Trop Biomed, 2011, 1(6): 439-443

15- Panneerselvam, C.; Ponarulselvam, S.; Murugan, K.; Kalimuthu, K. Thangamani, S,Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities. Asian Pac J Trop Biomed,2012, 574 -580.

16- Sulaiman, G M.; Mohammed, WH.; Marzoog, TR.; Al-Amiery ,A A.;Kadhum, A. H. ; Mohamad, A,Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using Eucalyptus chapmaniana leaves extract. Asian Pac J Trop Biomed, 2012, Accepted.

17- Dixit , S. N. and Tripathy , S. C. and upadyyey, R. R,The antifungal substance of rose flower (Rose indica) Economic Botany , 1976,. 30 : 371 – 373.

18- Jain, D., Kumar Daima, S., Kachhwaha, S and Kothari, S.L,Synthesis of plant mediated silver nanoparticles using Papaya Fruit Extract And Evaluation of their Antimicrobial Activities, Digest Journal of Nanomaterials and Biostructures, 2009, 4(3), 557-563.

19- Jian Xu, Xia Han, Honglai Liu, YingHu. “Colloids and Surfaces A,Physicochem”,. Eng Aspects, 2006, 273,179,

20- Sarah Ibrahim hashoosh, Ayad.M.A. Fadhil and Nabeel.k. Al-Ani, Production of Ag nanoparticles Using Aloe vera Extract and its Antimicrobial Activity. Journal of Al-Nahrain University Vol.17 (2), June, pp, 2014,165-171.

21 - Bhattacharya, R., and Mukherjee, P, Properties of ‘naked’ metal nanoparticles. Adv Drug Deliv Rev60, 2008 , 1289–1306 .

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22 - Salunkhe RB, Patil SV, Salunke BK ,

Larvicidal potential of silver nanoparticles synthesized using fungus,Cochliobolus lunatus against Aedes aegypti (Linnaeus, 1762) and Anopheles stephensiListo(Diptera: Culicidae). Parasitol Res, 2011, 109:823– 831.

23- Huang J, Li Q, Sun D, Su Y.X, Yang et al, Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnol, 2007, 18, 104-105.

24- Lee H.Y, Park H.K, Lee Y.M, Kim K, Park S.B0,Silver nanoparticles and its antibacterial evaluation for biomedical applications. Chem. Comm, 2007, 28, 2885.

25- Saxena A, Tripathi R.M., SinghR.P., Biological Synthesis of silver nanoparticles by using Onion (Allium cepa) extract and their antibacterial activity. Digest. J. Nanomater. Biostruct, 2010, 5, 427-432.

26- Khandelwal N, Singh A, Jain D, Upadhyay M.K., Verma H.N,Green synthesis of silver nanoparticles using Argimone mexicana leaf extract and Evaluation of their antimicrobial activities. Digest. J. Nanomater. Biostruct5, 2010, 483-489

27- Thirumurgan A, Tomy N.A, Jai Ganesh. R, Gobikrishnan S., Biological reduction of silver nanoparticles using plant leaf extracts and its effect an increased antimicrobial activity

28- Warisnoicharoen W, Hongpiticharoen P, Lawanprasert S, Alternation in Enzymatic function of Human cytochrome P450 by silver nanoparticles. Res. J. Environ. Toxicol,2001,5, 58-64.

29-Sereemaspun,A,Hongpiticharoen,P, Rojanath anes R, Maneewattanapinyo P, Ekgasit S, Warisnoi charoen W, Inhibition of human cytochrome P450 enzymes by metallic nanoparticles: A preliminary to. Int. J. nanogenomics Pharmacol, 2008, 4, 492-495