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RESEARCH ARTICLE

AZOLES EXPRESSED CYTO-PROTECTIVE EFFECTS TO HEPG2 CELLS

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ABSTRACT

Some azoles have proliferative effect on liver cells that showed it can cause angiogenesis as we observed in this study. In this study, HepG2 cells are used as liver cell line. As angiogenesis is the formation of new blood vessels, this drug can initiate the proliferation of more and more cells in liver which can lead to the regeneration of liver cells. This in-vitro study was based on one group of cells treated with our drugs of interest and other was untreated group. The study has shown increased proliferation of treated cells as compared to untreated group in MTT and antioxidant assays in dose dependant manner. As these drugs have proliferative effect, they also showed the increased angiogenesis than the normal cells. On the other hand they can act as antiapoptotic agent to reduce the apoptosis in some diseases like liver fibrosis. Antioxidant assays showed that these drugs played great role in reducing the oxidative stress that a cell can face during injury. SOD and GSH were the antioxidant enzymes which initially play their part in the reduction of superoxide ions. Superoxide ions are free radicals that cause damage to body cells, superoxide dismutase (SOD) and glutathione reductase (GSH) catalyze superoxide ions and convert them into molecular hydrogen and oxygen peroxide and show antioxidant defense response towards damaging free radicals. Thus end results showed that these azoles can have proliferative and regenerative effects on liver cells.

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INTRODUCTION

Human liver cell lines have been considered as most suitable for pharmaceutical and toxicological experiments for drug discovery. In in-vitro studies, HepG2 cells and primary hepatocytes have been proved useful. With comparison to primary hepatocytes, HepG2 cells are easy to handle and have high proliferative capacity than primary hepatocytes (Wilkening, Stahl *et al*. 2003). Omeprazole is a proton pump inhibitor, firstly reported by Gugler and Jensen and widely used for cure of peptic ulcers. It is metabolized by CYP2C19 and CYP3A4. Former enzyme convert it into hydroxyomeprazole and latter enzyme into omeprazole sulphone. Omeprazole also induces CYP1A2. Omeprazole clears out other drugs from the body which may be harmful. In *Helicobacter pylori* infected patients, omeprazole proved significantly better result as compared to normal group (Bibi 2008). Omeprazole is a member of heterocyclic aromatic organic compounds (benzimidiazoles) (Massoomi, Savage *et al*. 1993) also known as proton pump inhibitor, is commonly used as effective treatment for all upper gastrointestinal ailments such as Gastroesophageal reflux disease (GERD) as well as in peptic ulcers (Sachs, Shin *et al*. 1993, Shin and Sachs 2004).

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American college of gastroenterology guidelines also suggested omeprazole as a therapy for GERD. Acid suppression with omeprazole is so much effective in reducing pain, heartburn and regurgitation (DeVault, Castell *et al*. 2005). This drug acts by inhibiting the H+/K+ ATPase to prevent the acid production . As a result, Omeprazole raised pH of the stomach lumen, a step thought to be important in the therapy for the treatment of gastritis (local infection or inflammation related to gastric mucosa). In some studies it has been shown that GERD is common among patients with asthma. A double blind study was used to determine that suppression of GERD with Omeprazole can improve the pulmonary function (Meier, McNally *et al*. 1994). Fluconazole is preferable over ketoconazole due to many reasons such as it is favourite in many ailments like cryptococcal meningitis, disseminated candidiasis, coccidioidomycosis, chronic mucocutaneous candidiasis, genito-urinary, peritoneal, vaginal, oesophageal, oropharyngeal candida infections. It is safe upto 1600 mg dose and does not affect cortisol and testosterone producction. It has fewer drug interactions (Maertens 2004). It is easily excreted (70-80%) via urine (Humphrey, Jevons *et al*. 1985) and can cross blood-brain barrier (Arndt, Walsh *et al*. 1988). It is highly soluble in water and can be given parentally. That's why, it is safe as oral treatment due to maximum absorption and is not affected by gastric pH (Brammer, Farrow *et al*. 1990). Voriconazole is structurally related to fluconazole

(Sabo and Abdel-Rahman 2000). It is effective against a number of moulds and fungi such as *Trichosporon*, *Acremonium*, *Scedosporium*, *Penicillium*, *Fusarium* spp., dermatophytes, scedosporiosis, fusariosis, dimorphic fungi, *Candida neoformans*, *Aspergillus* spp. and *Candida* spp. Due to resistance in zygomycetes, it was found ineffective. It can easily cross blood-brain barrier. Voriconazole especially proved useful in treating oropharyngeal candidiasis in patients suffering from cerebral aspergillosis, oesophageal candidiasis and AIDS. But it has also side effects too especially it affects liver (Potoski and Brown 2002). It also affected physiology of eyes in 10% patients (Maertens 2004). The aim of this study was to evaluate the effects of these azoles on liver cell line which showed that azoles can initiate the proliferative effects on liver cells that will be a new approach in future studies to further check or evaluate the effect of this drug on liver cells.

MATERIALS AND METHODS

Sampling of HepG2 cell line: HepG2 cell line was preserved in cryo vials in liquid nitrogen in cell culture lab in The University of Lahore. That cryovials were revived for further processing.

Availability of azoles: The drugs of interest were obtained from medical store in the form of capsules or pellets.

Culturing of HEPG2 Cell Line: The cryo vials saved in liquid nitrogen cylinder was thawed. In culturing flask, HepG2 cell line was added along with DMEM-HG, fetal bovine serum (FBS). Antibiotics like gentamycin, streptomycin and penicillin were added to the culturing flask for the safe and proper cultivation of cell line. When the cells achieved 70-80% confluence, their splitting was conducted. Splittingof cells was done by washing the walls of the culturing flask (to which the cells were attached) with normal saline and incubated with trypsin-EDTA until the cells fully detached from the surface of culturing flask. The detachment was confirmed by observing flask under microscope. FBS was added and mixture was centrifuged at 3000rpm for 5 mints by adding the mixture in 15ml tube. After centrifugation supernatant was removed and pellet was re-platted.

Treatment of Hepg2 Cell Line with Azoles: 96-well plate was cultured with HepG2 cell line for cell proliferation. Then HepG2 cell line was divided into 2 groups, one group was untreated and other was treated with azoles. Cultured cells were taken for treatment. After 24 hours of treatment, 96-well plate was used for cell proliferation assays and the harvested media taken from all experimental groups was used for biochemical assays for further evaluation.

Cell Proliferation Assay: To evaluate the proliferative potential MTT 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide was performed on cultured 96-well plate. MTT assay was used to evaluate the cytotoxicity of cells, or to check the viability of cells. Monolayer of cells was washed with phosphate buffer saline (PBS), then incubated 100µl complete medium containing 25µl of MTT solution for 2 hours. MTT was converted into purple colored compound formazan by the enzyme (NADPH dependant Oxidoreductase) released from mitochondria when the cells are viable. To solubilize formazan crystals 10 % SDS (sodium dodecyl sulphate) was added. The absorbance was taken at 570nm.

Enzyme Linked Immunosorbent Assay (ELISA): Solid phase sandwich ELISA was performed for HepG2 cell lines for VEGF. Numbers of wells were determined in 96-well plate and 100µl of captured antibody in coating buffer was added to each well. Plate was then incubated for 24 hours and then washed with washing solution (each well 3 times).after washing the wells 200µl of blocking solution was added to each well and plate was incubated for 30 minutes. After removing the washing solution, each well was again washed 3 times. Samples of desired concentrations were diluted in sample diluents and 100µl of standard was transferred into each well. After 6 hours incubation the wells were again washed 3 times. 100µl of HRP conjugate diluents was transferred to each well and plate was incubated overnight. At the end TMB was used as substrate solution and 100µl of it was transferred to each well. After incubating the plate for 15-20 minutes each well was loaded with 100µl of H_2SO_4 as stop solution. The absorbance was taken at 450nm and the graph was plotted.

Evaluation of Antioxidative Enzymes

Superoxide Dismutase (sod): Superoxide dismutase activity was done on HepG2 cell line and measured in a 96-well plate. The 250µl of reaction mixture was prepared with 25µl of KH_2PO_4, 56.5µl of NBT, 5µl of EDTA, 32.5µl of methionine, 10µl of riboflavin. Then 121µl of sample obtained from different experimental groups of HepG2 was added. After 10 minutes of exposure of light the absorbance was taken at 570nm. The graph was plotted.

Glutathione Reductase Assay (GSH): GSH assay was performed in 96-well plate with a reaction mixture of 200µl in each well. The reaction mixture was prepared by mixing 20µl KH_2PO_4 , 40µl of EDTA, 20µl of NADPH, 10µl of oxidized glutathione. The sample obtained from different experimental groups of HepG2 cell lines were added (100µl) with 10µl of water. After 3 minutes of exposure to light the absorbance was taken at 340nm and the graph was plotted.

RESULTS

Increased Proliferative Activity of Azoles: In this assay it had been determined that there were different proliferation rates when treating the cells with different amount of drug doses. When the cells were treated with different doses of omeprazole such as 10 mg/ml to 100 mg/ml, different proliferative effects were observed. 10 mg/ml (91.66%), 20 mg/ml (116.66%), 30 mg/ml (112.5%), 40 mg/ml (108.33%), 50 mg/ml (275%) and 100 mg/ml (316.66%) were observed. Voriconazole increases the cell proliferation at different doses treatment for 24 hours, which later shows the significant increase in cell number. 10 mg/ml (277.77%), 20 mg/ml (555.55%), 30 mg/ml (833.33%), 40 mg/ml (1111.11%), 50 mg/ml (1388.88%) and 100 mg/ml (2777.77%) were observed. Liver cells were treated with the drug of our interest, Fluconazole at different doses 10 to 100 mg/ml. When results were plotted against the controlled or untreated group of cells, it was observed that there was a significantly higher proliferation at the dose of 100 mg/ml. 10 mg/ml (111.11%), 20 mg/ml (222.22%), 30 mg/ml (333.33%), 40 mg/ml (444.44%), 50 mg/ml (555.55%) and 100 mg/ml (1111.11%) were observed as shown in (Fig: 1).

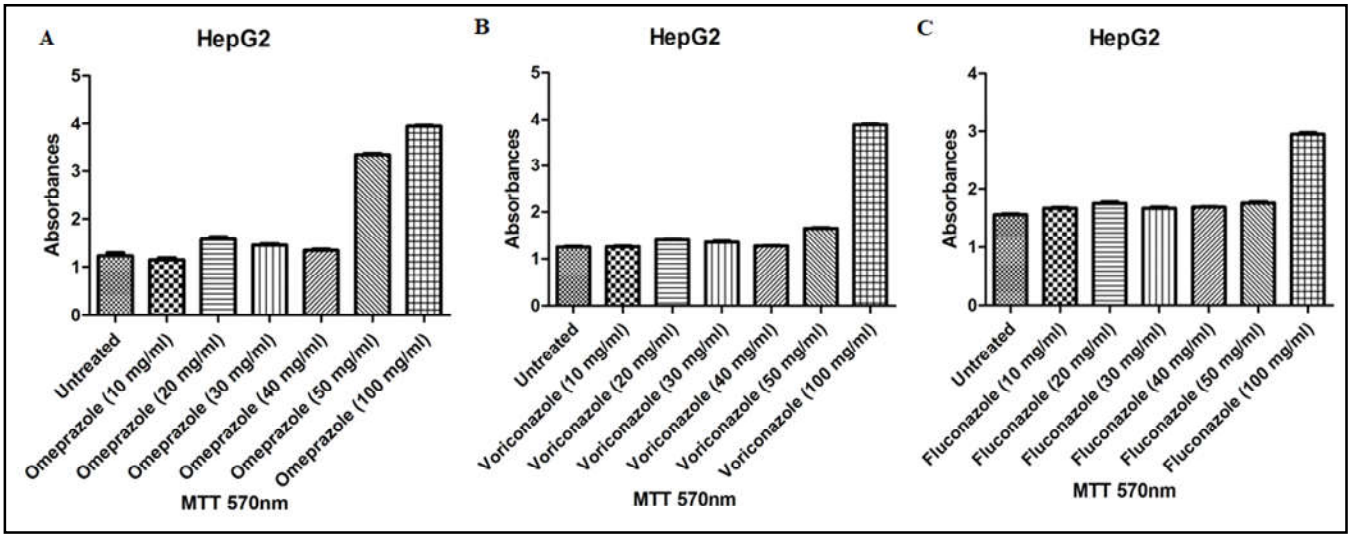

Figure 1. Proliferation rates of HepG2 cells after treating with Omeprazole, voriconazole and fluconazole between untreated or treated groups where HepG2 cells are treated with azoles at different amount of doses which is showing the different rates of proliferation

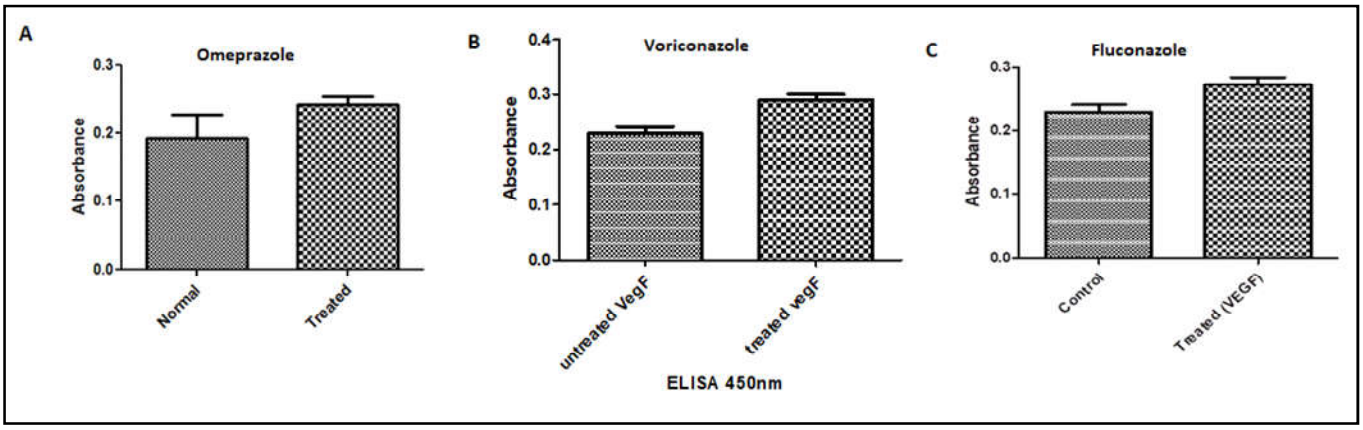

Figure 2. Rate of angiogenesis after treating cells with Omeprazole, voriconazole and fluconazole through ELISA assay where normal group is untreated, treated group release high level of VEGF in treated media with omeprazole. Values taken were expressed as mean ± SEM

At 100 mg/ml dose of all azoles, significantly higher proliferation was observed when compared with the controlled group of cells, Omeprazole (316.66%), Voriconazole (2777.77%) and Fluconazole (1111.11%) as shown in the (Fig: 1). Maximum dose of 100mg/ml for every azole is selected for further experiments.

Elisa (Enzyme linked Immunosorbent Assay) for Azoles: ELISA assay was performed to check angiogenesis and apoptosis of cells between normal cells media and cells media treated with Omeprazole. The increased expression of VEGF in treated media showed that there was increased level of angiogenesis (0.242±0.0107) than the normal cells (0.193±0.0339). Increased level of angiogenesis was observed (125.388%) (Fig: 2). ELISA was performed after the treatment of the cells with voriconazole. Absorbance was taken at 450nm and the results showed the increased levels of angiogenesis in the treated cells (0.282± 0.006) with voriconazole as compared to the untreated cells (0.232±0.006). Increased level of angiogenesis was observed (121.55%) (Fig: 2). After treating hepG2 cells with Fluconazole comparatively high levels of angiogenesis were seen via ELISA. So, according to our results treatment of cell line by Fluconazole increases the level of angiogenesis. As VEGF is the major factor of angiogenesis, its level was increased in the cell lines when treated with Fluconazole (0.258±0.009) as compared to pre-treated cells (0.237±0.013) as shown in the figure (108.86%) (Fig: 2).

ELISA was performed for evaluating the apoptosis level by using p53 antibody. The p53 expression observed in cells treated with Omeprazole was low (0.108±0.0480), indicating apoptosis. While in case of normal (0.243±0.0183) its activity was high. Reduced level of p53 was observed (44.44%) (Fig: 3). After treatment of cells with Voriconazole, absorbance was taken at 450 nm and the results showed the low levels of apoptosis in the treated cells (0.243± 0.0183) with Voriconazole as compared to the untreated cells (0.111± 0.0493) (45.67%) (Fig: 3). HepG2 cell lines when treated with Fluconazole showed low levels of apoptosis (0.109±0.037) as compared to pre-treated cells (0.243±0.027) as shown in the figure (44.85%) (Fig: 3).

Antioxidants by Azoles: In the evaluation of anti oxidative enzymes glutathione reductase assay (GSH) and superoxide dismutase (SOD) assay was performed between untreated and treated cells by azoles dose of 100 mg/ml each. After treating cells with Omeprazole, medium collected from all treatment was analyzed for SOD activity. It was observed that SOD activity was decreased in treated cells (0.399±0.00924), whereas activity in untreated cells was increased (0.845±0.0278) (47.21%) (Fig: 4). When SOD was performed on the treated cells with voriconazole relatively low levels were seen (0.121±0.065) as compared to controlled group (0.239±0.019) (50.62%).

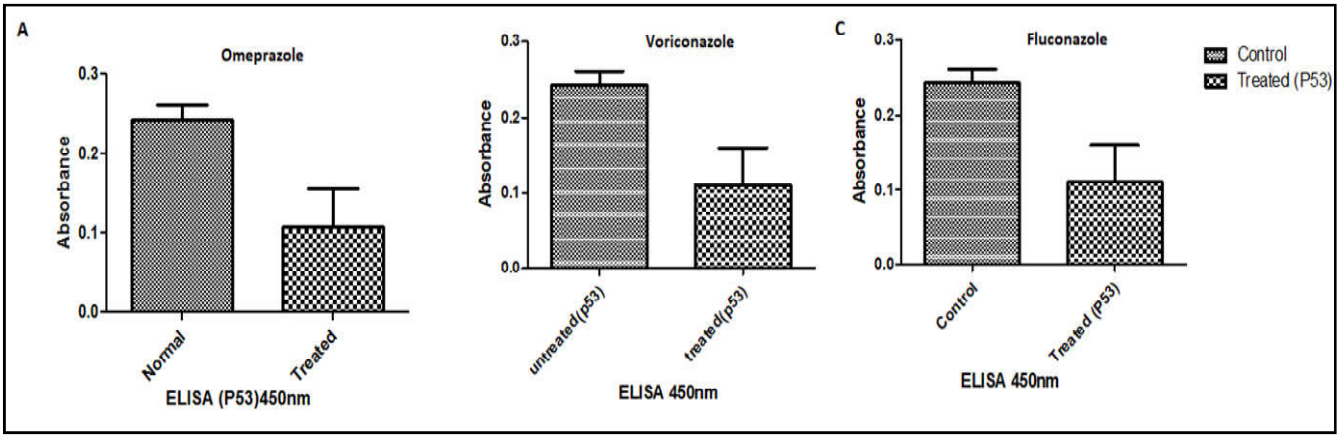

Figure 3. Rate analysis of dead cells between normal and treated cells with p53 as normal group is untreated HepG2 cells in plane medium and cells treated with omeprazole, voriconazole and fluconazole showed the low level of p53 results in decreased level of dead cells. Values were taken as mean ± SEM

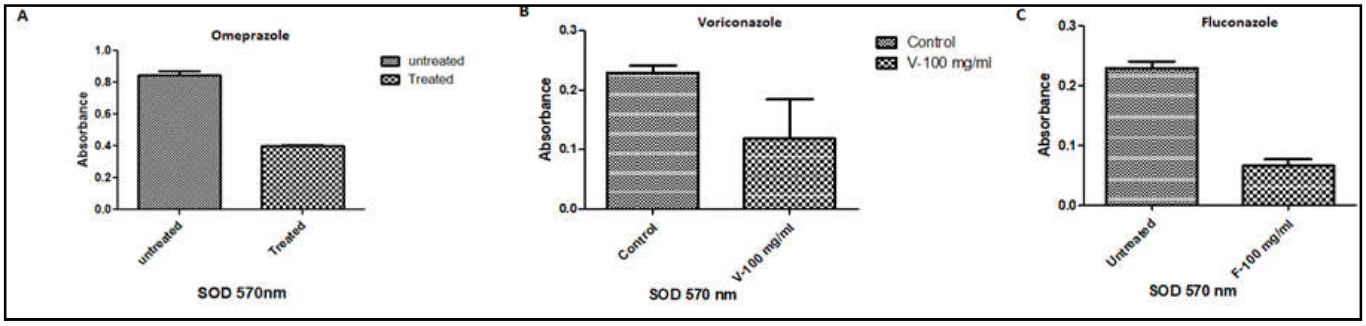

Figure 4. SOD analysis between treated or untreated cells indicated that cells are in less oxidative stress as superoxide dismutase also contribute its part in reducing stress in case of damage and any injury at some point in dose dependant manner, as it can cause reduction of superoxide radicals (that can cause damage) into either free ordinary oxygen or hydrogen peroxide. Values were taken as mean ± SEM, which shows the significant difference between treated and untreated cells

Low levels of SOD showed that less ROS were produced in the presence of the drug Voriconazole (Fig: 4). After treating cells with fluconazole and the medium collected from a treatment was analyzed for SOD activity separately. SOD activity was decreased in the presence of Fluconazole (0.061±0.008) indicating low levels of reactive oxidants species and stress after the treatment as compared to control (0.238±0.011) as shown in the figure (25.63%) (Fig: 4).

Glutathione reductase activity (GSH): After treating the HepG2 cells with Omeprazole, GSH activity was analyzed. It was observed that GSH activity was high in treated cells (1.05±0.221), indicating the oxidative stress after treatment. Whereas in that case of untreated (0.399±0.00924) its activity was decreased (263.15%) (Fig: 5). When this assay was conducted on the treated cells with Voriconazole, relatively low levels were seen (0.201±0.080) as compared to controlled group (0.237±0.008). Low levels of GSH showed that less stress was produced in the presence of the drug voriconazole (84.81%) (Fig: 5). After treating cells with Fluconazole and the medium collected from a treatment was analyzed for GSH activity separately. GSH activity was increased in the presence of fluconazole (3.38±0.001) indicating low levels oxidative stress after the treatment as compared to control (0.2±0.002) as shown in the figure (1690%) (Fig: 5).

DISCUSSION

The major hypothesis of this study was that fluconazole, voriconazole and omeprazole may have a proliferative effect in liver cells. To check the proliferation of these azoles, MTT assay was done in which cell revival was detected. HepG2 cell lines were cultured, azoles were given to cells and after the 24 hr treatment the treated media of those cells were taken. There are some compounds that play role in treating liver fibrosis (You, Wang *et al*. 2000), these azoles are also among that compounds as they reduced the cell death. Antioxidants are the source for first line defense against free harmful radicals in body that can damage the chemical reactions in body or cells. Antioxidants regulation is dependent on the state of a cell oxidative stress (Rodriguez, 2004). In antioxidant assay we evaluated superoxide dismutase and glutathione reductase enzymes to observe the oxidative stress of cells that were injured. SOD and GSH both showed their role in reducing the oxidative stress of cells by removing the superoxide free radicals. They catalyzed the harmful radicals to convert them in molecular hydrogen and oxygen ions in body thus showed their role in the regeneration of cells followed by different doses. Enzyme linked immunosorbent assay showed the VEGF high level with the group of cells that were treated with omeprazole. Cells treated with omeprazole showed a high level of VEGF that is a vascular endothelial growth factor which is a great factor and protein made by cells that further initiate or stimulate the formation of new blood vessels from existing vessels. The results showed that omeprazole has some factors which can actually cause angiogenesis but further studies are needed for confirmation. Voriconazole is a secondary trizole antifungal drug and is the derivative of fluconazole with its improved activity against invasive aspergillosis (a serious fungal infection caused in the lungs of immunocompromised patients) and is also used in the treatment of other dangerous fungal infections.

After treating the cultured cells with voriconazole at the dose of 100mg/ml, MTT assay was applied on them to check the viability and the proliferation. After taking the absorbance at 570nm, results showed high rates of proliferation in the treated cells. ELISA is easy to perform and its reagents are inexpensive comparatively. Several kinds of ELISA like Sandwich ELISA, direct ELISA, competitive ELISA, and indirect ELISA. ELISA all of them employ clorometric enzymes and antibodies (Gan and Patel 2013). Treated liver cell line with voriconazole was checked with ELISA to evaluate the levels of angiogenesis and apoptosis by using VEGF and p53 antibodies. After taking the absorbance at 450nm the levels of angiogenesis were higher as compared to apoptosis in the treated cells. Hence, voriconazole has the potential to increase the angiogenic activity of the cells. In addition, to check out the activity of antioxidants (GSH) glutathione reductase assay and (SOD) superoxide dismutase assay was performed on the treated cell line. Relatively low levels of both antioxidants were observed showing less stress levels. After the results study depicted that voriconazole holds the property of proliferation in liver cell line. Higher rates of angiogenesis and proliferation can lead to the cancer more effectively and they can promote the cancerous cells. Hence, it cannot be given to the patients suffering from cancer. Fluconazole has been used for its anti-fungal activity from a long period of time. Synthetic azole antifungals such as fluconazole, were introduced 40 years ago in 1990s and are still a first-lineantifungal agent for the treatment and prophylaxis of various fungal problems such as invasivecandidiasis in children and infants (Pappas, Kauffman *et al*. 2009, Spitzer, Griffiths *et al*. 2011, Hope, Castagnola *et al*. 2012).

Right after the culturing and treatment of liver cells by fluconazole at the doses of 10mg/ml and 100 mg/ml for 24 hours MTT proliferative assay was performed and the formazam crystals were solubilized with the detergent SDS. Proliferation was detected on both doses. Every time whenever absorbance was taken, higher rates of proliferation were seen on 100mg/ml than the 10 mg/ml. ELISA was performed in the presence of fluconazole to check the angiogenesis and apoptosis levels on liver cells. For evaluating the apoptosis levels p53 antibody was used and absorbance was taken at 450nm. Results efficiently supported the proliferative observations by showing the less apoptosis rates when compared with angiogenesis. In the presence of fluconazole when angiogenesis levels were measured by using VEGF antibody the results showed comparatively higher rates of angiogenesis. Furthermore, in the treated group with fluconazole for the detection of ROS (reactive oxidant species) Glutathione reductase assay and superoxide dismutase assay were performed. The levels of both antioxidants were different, as levels of GSH were high whereas SOD was comparatively low.

Conclusion

All the assays evaluated, have shown that these azoles that were tested, have proliferative effects on liver cell which indicate that these drugs can be useful for liver cell proliferation or injury protection in future.

REFERENCES

Arndt, C. A., T. J. Walsh, C. L. McCully, F. M. Balis, P. A. Pizzo and D. G. Poplack. 1988. "Fluconazole penetration into cerebrospinal fluid: implications for treating fungal infections of the central nervous system." The Journal of infectious diseases 157(1): 178-180.

Bibi, Z. 2008. "Role of cytochrome P450 in drug interactions." Nutrition & metabolism 5(1): 27.

Brammer, K., P. Farrow and J. Faulkner, 1990. "Pharmacokinetics and tissue penetration of fluconazole in humans." Reviews of infectious diseases 12 (Supplement_3): S318-S326.

DeVault, K. R., D. O. Castell and G. American College of 2005. "Updated guidelines for the diagnosis and treatment of gastroesophageal reflux disease." *Am J Gastroenterol* 100(1): 190-200.

Gan, S. D. and K. R. Patel 2013. "Enzyme immunoassay and enzyme-linked immunosorbent assay." *J Invest Dermatol* 133(9): e12.

Hope, W. W., E. Castagnola, A. H. Groll, E. Roilides, M. Akova, M. C. Arendrup, S. Arikan-Akdagli, M. Bassetti, J. Bille, O. A. Cornely, M. Cuenca-Estrella, J. P. Donnelly, J. Garbino, R. Herbrecht, H. E. Jensen, B. J. Kullberg, C. Lass-Florl, O. Lortholary, W. Meersseman, G. Petrikkos, M. D. Richardson, P. E. Verweij, C. Viscoli and A. J. Ullmann, 2012. "ESCMID* guideline for the diagnosis and management of Candida diseases 2012: prevention and management of invasive infections in neonates and children caused by Candida spp." *Clin Microbiol Infect* 18 Suppl 7: 38-52.

Humphrey, M., S. Jevons and M. Tarbit, 1985. "Pharmacokinetic evaluation of UK-49,858, a metabolically stable triazole antifungal drug, in animals and humans." *Antimicrobial agents and chemotherapy* 28(5): 648-653.

Maertens, J. 2004. "History of the development of azole derivatives." *Clinical Microbiology and Infection* 10: 1-10.

Massoomi, F., J. Savage and C. J. Destache, 1993. "Omeprazole: a comprehensive review." Pharmacotherapy: *The Journal of Human Pharmacology and Drug Therapy* 13(1): 46-59.

Meier, J. H., P. R. McNally, M. Punja, S. R. Freeman, R. H. Sudduth, N. Stocker, M. Perry and H. S. Spaulding, 1994. "Does omeprazole (Prilosec) improve respiratory function in asthmatics with gastroesophageal reflux? A double-blind, placebo-controlled crossover study." Dig Dis Sci 39(10): 2127-2133.

Pappas, P. G., C. A. Kauffman, D. Andes, D. K. Benjamin, Jr., T. F. Calandra, J. E. Edwards, Jr., S. G. Filler, J. F. Fisher, B. J. Kullberg, L. Ostrosky-Zeichner, A. C. Reboli, J. H. Rex, T. J. Walsh and J. D. Sobel, 2009. "Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America." Clin Infect Dis 48(5): 503-535.

Potoski, B. A. and J. Brown, 2002. "The safety of voriconazole." Clinical infectious diseases 35(10): 1273-1275.

Sabo, J. A. and S. M. Abdel-Rahman, 2000. "Voriconazole: a new triazole antifungal." *Annals of Pharmacotherapy* 34(9): 1032-1043.

Sachs, G., J. M. Shin, M. Besancon and C. Prinz, 1993. "The continuing development of gastric acid pump inhibitors." Aliment Pharmacol Ther 7 Suppl 1: 4-12, discussion 29-31.

Shin, J. M. and G. Sachs 2004. "Differences in binding properties of two proton pump inhibitors on the gastric H+, K+-ATPase in vivo." *Biochem Pharmacol* 68(11): 2117-2127.

Spitzer, M., E. Griffiths, K. M. Blakely, J. Wildenhain, L. Ejim, L. Rossi, G. De Pascale, J. Curak, E. Brown, M. Tyers and G. D. Wright 2011. "Cross-species discovery of syncretic drug combinations that potentiate the antifungal fluconazole." *Mol Syst Biol* 7: 499.

Wilkening, S., F. Stahl and A. Bader, 2003. "Comparison of primary human hepatocytes and hepatoma cell line Hepg2 with regard to their biotransformation properties." *Drug metabolism and disposition* 31(8): 1035-1042.

You, H., B. Wang and T. Wang, 2000. "[Proliferation and apoptosis of hepatic stellate cells and effects of compound 861 on liver fibrosis]." Zhonghua Gan Zang Bing Za Zhi 8(2): 78-80.

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