Antihyperglycemic and antiadhesion activity of gallic acid in induced urinary tract infection in diabetic rats

N. A. Mohammed Ali, Sh. Z. Saeed and S. Noori
College of Medicine\ Hawler Medical University, Erbil, Iraq

Abstract

This study was undertaken to investigate the role of gallic acid in attenuating hyperglycemia and inhibiting bacterial adhesion to bladder epithelial cells in induced diabetes and urinary tract infection in rats. Diabetes was induced in rats by intraperitoneal injection of alloxan. Blood glucose levels were measured daily and glucose levels of >250 mg/dl was considered as positive for diabetes initiation. Bladder infection was induced by transurethral catheterization of *Escherichia coli*. Gallic acid was administered orally at a dose of 2.5,5 and 10 mg/kg and compared with a control group and a group administered ciprofloxacin orally (7.2 mg/kg). Blood glucose level was measured after induced *E.coli* infection in the rats of each group then the rats were sacrificed and the bladders were taken for histopathology and for the determination of adhesion of *E.coli* to bladder epithelium. The study demonstrated that gallic acid at concentrations of 2.5, 5 and 10 mg/kg significantly ($P \leq 0.05$) reduced elevated blood sugar and inhibited the adhesion of *E.coli* to bladder epithelial cells compared to controls. Histological changes revealed decrease in severity of acute inflammation and edema with decreased number of neutrophils invading epithelial cells compared to diabetic group.

Key words: Gallic acid, hyperglycemia, *E.coli*, bladder epithelial cells, adhesion
E. mail: nabdulqader@yahoo.co.uk

فعالية حمض الكاليك في خفض فرط سكر الدم ومنع الالتصاق في الجرذان المستحدثة فيها مرض السكري والتهاب المجاري البولية

نضال عبد القادر محمد علي، شذى زكر سعيد وسردار نوري
كلية الطب/ جامعة هولير الطبية/ أربيل

الخلاصة

صممت هذه الدراسة للبحث عن دور حمض الكاليك في خفض فرط كلوكوز الدم ومنع الالتصاق الجرثومي بالخلايا الظهارية للمثانة في الجرذان المستحدثة فيها مرض السكري والتهاب المجاري البولية. تم استحداث مرض السكري في الجرذان بإعطائها مادة الالوكسان عن طريق البريتون. قيس مستوى الكلوكوز في الدم يوميا وتم اعتبار مستوى الكلوكوز اكثر من 250 ملغم/ دسي ليتر إشارة لحدوث السكري. تم استحداث الإصابة في المثانة عن طريق تسريب جرثومة الايشيريكيا القولونية من خلال الإحليل. تم إعطاء حمض الكاليك عن طريق الفم وبجرعة 2.5، 5 و10 ملغم/ كغم من وزن الجسم وتم مقارنتها مع مجموعة سيطرة ومجموعة أعطيت عقار السبروفلوكساسين بجرعة 7.2 ملغم/ كغم. تم قياس مستوى الكلوكوز بعد استحداث الإصابة في كل جرذ في المجاميع المختلفة وبعدئذ تم قتل الجرذان وأخذت المثانة للتقطيع النسيجي وتم جمع الخلايا الظهارية لمعرفة عدد جرثومة الايشيريكيا القولونية الملتصقة بالخلايا الظهارية للمثانة. أظهرت الدراسة ان حمض الكاليك المعطى بجرعة 2.5، 5 و10 ملغم/ كغم خفض معنويا فرط جلوكوز الدم بمستوى 0.05 وثبط التصاق جرثومة الايشيريكيا القولونية بالخلايا الظهارية مقارنة بمجموعة السيطرة. بينت نتائج فحوصات التغييرات النسيجية تناقص في حدة الالتهاب الحاد والوذم الالتهابي مع تناقص اعداد خلايا الدم البيضاء العدلة التي اجتاحت الخلايا الظهارية للمثانة مقارنة مع مجموعة المستحدثة فيها مرض السكري.

الكلمات المفتاحية: حمض الكاليك، فرط كلوكوز الدم، الايشيريكيا القولونية، الخلايا الظهارية، الالتصاق.

Introduction

Diabetes mellitus (DM), together with its various complications, is becoming a serious threat to human health. DM is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, action or both leading to cardiovascular diseases, and renal failure (1). It is widely accepted that hyperglycemia generates reactive oxygen species (ROS) which in turn contribute to pancreatic cell or tissue damage and dysfunction both in type 1 and 2 diabetes (1, 2, 3). Patients with diabetes mellitus are at increased risk of infections, with the urinary tract being the most frequent infection site. Factors that enhance risk of urinary tract infections in diabetic patients are poor metabolic control, impairments in the immune system and incomplete bladder emptying due to autonomic neuropathy (4, 5). These infections are mostly caused by resistant pathogens and often diabetic patients experience chronic or recurrent urinary tract infection that are resistant to antimicrobial agents (6, 7, 8). Adhesion is necessary for *E.coli* to bind to uroepithelial cells (9). This process occur through expression of particular virulence factors that specifically enhance their ability to cause infections of the urinary tract. These factors include numerous fimbriae (called adhesins) that diffuses on bacterial cell surface, enable the bacteria to recognize host receptors, attach to the urinary tract mucosa and trigger signals to start the disease process (9, 10). Therefore, interference of these virulence factors is an attractive approach to manage bacterial infection (10). The use of natural products is stepping up in diabetic patients, and continuous search for compounds with hypoglycemic effects, along with management of diabetic complications is receiving more attention (11). Gallic acids (GA) is a compound of plant origin, commonly found as free or bound form in tea, nuts, fruits, beverages and spices (12). GA has been shown to possess various pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and anti-cancer activities (12, 13, 14). Gallic acid, as derivatives of phenolic acid is known to be toxic to micro-organisms as bacteria, fungus and viruses and many studies showed that the use of gallic acid provides an economic approach in the treatment of microbial infections (15, 16, 17). Researches focused on interference of bacterial adhesion by medicinal plants in *in vitro*, and *in vivo* studies and clinical trial of antiadhesive effects of cranberry fruit on uropathogenic *E.coli* are recommended (18). Diabetic patients with recurrent urinary tract infection fails to respond to conventional antimicrobial agents. Therefore, this study was intended to investigate the hypoglycemic activity of gallic acid and the anti-adhesion of *E. coli* to urinary epithelium in diabetic-induced rats.

Materials and Methods

Female albino rats (n=30) with body weight between 200-250 gram were used in the experiment. Pellets for rats feeding and water were available *add libitum* and they were kept in plastic cages at room temperature of 20-25°C. The rats were divided into two groups; Group A (5 rats) was considered control non-diabetic non-treated group received only distilled water and group B (25 rats) was considered diabetics. Group B rats were fasted for 12 hours thereafter injected IP with freshly prepared alloxan monohydrate in distilled water once daily at a dose rate of 135 mg/kg for 7 days (19). Blood glucose level was measured daily with a glucometer using blood sample from the tail vein of the rats after two hours of the IP injection of alloxan to monitor induction of hyperglycemia for a period of one week. Blood glucose levels of >250 mg/dl was considered positive for diabetes initiation (19). Group B was subdivided equally to 5 subgroups. Subgroup 1, considered diabetic-infected, non-treated received only distilled water. The second, third

and fourth subgroups were considered diabetic treated groups administered gallic acid orally daily either at dose of 2.5, 5, 10 mg /kg respectively and the fifth subgroup was given ciprofloxacin orally at 7.2 mg/kg. Bladder infection was induced according to the method of (20). The rats were anesthetized by IP injection of a mixture of ketamine (100 mg/kg) and xylazine (35 mg/kg). Thereafter the urethral region was disinfected with alcohol (70%) and one milliliter of bacterial suspension containing 1×10^6 *Escherichia coli* prepared freshly in phosphate buffer from an overnight broth was infused slowly into the bladder of the anesthetized rats using a blunt slightly bent needle over 30 seconds period. Urine samples were collected after 72 hours of infection from each rat by gentle massage and pressing bladder region and examined for bacteriuria and cystitis by culturing on MacConkey agar for confirmation of infection. After, 18 hours of confirmed urinary infection, gallic acid was administered orally at a dose of either 2.5, 5, 10 mg/kg to the treatment groups once daily. Doses of gallic acid were chosen according to *in vitro* susceptibility testing of antimicrobial activity and MIC of gallic acid against *E.coli* ATCC 252922 performed prior to experimental infection according to Clinical and Laboratory Standards Institute (21). After one week of gallic acid administration, blood glucose levels were measured, then bladder epithelial cells were scratched from three rats, placed onto clean slides, fixed with alcohol and stained with methylene blue and processed for the determination of *E.coli* adhesion to the bladder epithelium by counting the number of *E.coli* attached to 50 epithelial cell according to the method of (22). The urinary bladder was excised from the remaining two rats of each group for histopathology, fixed in 4% neutral formaldehyde, embedded in paraffin blocks. The sections (4-5 µm) were stained with Hematoxylin and Eosin, and were analyzed for histopathological studies. Statistical analysis was performed using the SPSS software (Version 20 for windows). Differences between groups were analyzed by one-way analysis of variation (ANOVA). A *P* value of 0.05 was considered statistically significant differences.

Results

The mean blood glucose levels in normal control and all experimental groups are shown in (Table 1).

Table (1) Mean concentration of blood glucose (mg/dl) and % adhesion of *E.coli* to bladder epithelial cells of rats in different experimental groups

Group	Control/ non diabetic	Diabetic	Gallic acid			Ciprofloxacin
			2.5 mg/kg	5 mg/kg	10 mg/kg	7.2 mg/kg
Glucose ± SD	116.8 ±5.2 a	193±3.2 b	133.8±8.5 c	122.6±9.3 ad	118.8±4.7 ad	189.2±6.4 be
Adhesion± SD	-	100 a	69 b	43 c	36 d	8 e

Different letters indicate statistical differences at P≤ 0.05 between groups

There was a significant ($p\leq0.05$) increase in the mean blood sugar level in the diabetic group rats compared to the normal control group (Table 1). Gallic acid at doses of (2.5, 5 and 10 mg/Kg) showed a significant ($p\leq0.05$) decrease in mean blood glucose levels compared to diabetic group. The mean blood glucose levels of diabetic rats administered gallic acid at 5 and 10 mg/kg were statistically not significantly ($P>0.05$) different than those of control non-diabetic group however, there was a statistical significant ($p\leq0.05$) differences between the mean blood glucose levels of control group and diabetic rats administered gallic acid at 2.5 mg/kg. Although the mean blood glucose levels of ciprofloxacin-treated diabetic rats was significantly ($P\leq0.05$) higher than the controls

however, it was statistically not significantly (P>0.05) different than those in the diabetic rats. Adhesion of *E.coli* to bladder epithelial cells, was significantly (p≤0.05) reduced after administration of gallic acid at doses of 2.5, 5 and 10 mg/kg and ciprofloxacin compared to diabetic infected not-treated rats (Table 1). Histopathological changes (Fig. 1) revealed in group A (Non-diabetic non-infected); normal morphology with no inflammatory change. While in group B, changes were according to subgroups: In subgroup 1 (Diabetic non-treated) showed severe acute inflammation, severe edema, mixed inflammatory cells infiltration mainly neutrophils invading epithelial layer. In subgroups 2, 3 and 4 (Diabetic gallic acid treated): with increasing dose of gallic acid, a decrease in severity of acute inflammation and edema with decreased number of neutrophils invading epithelial cells were observed. While in subgroup 5 (Diabetic ciprofloxacin treated); mild-moderate edema and few mixed inflammatory cells infiltration with no neutrophil invading epithelial layer were observed.

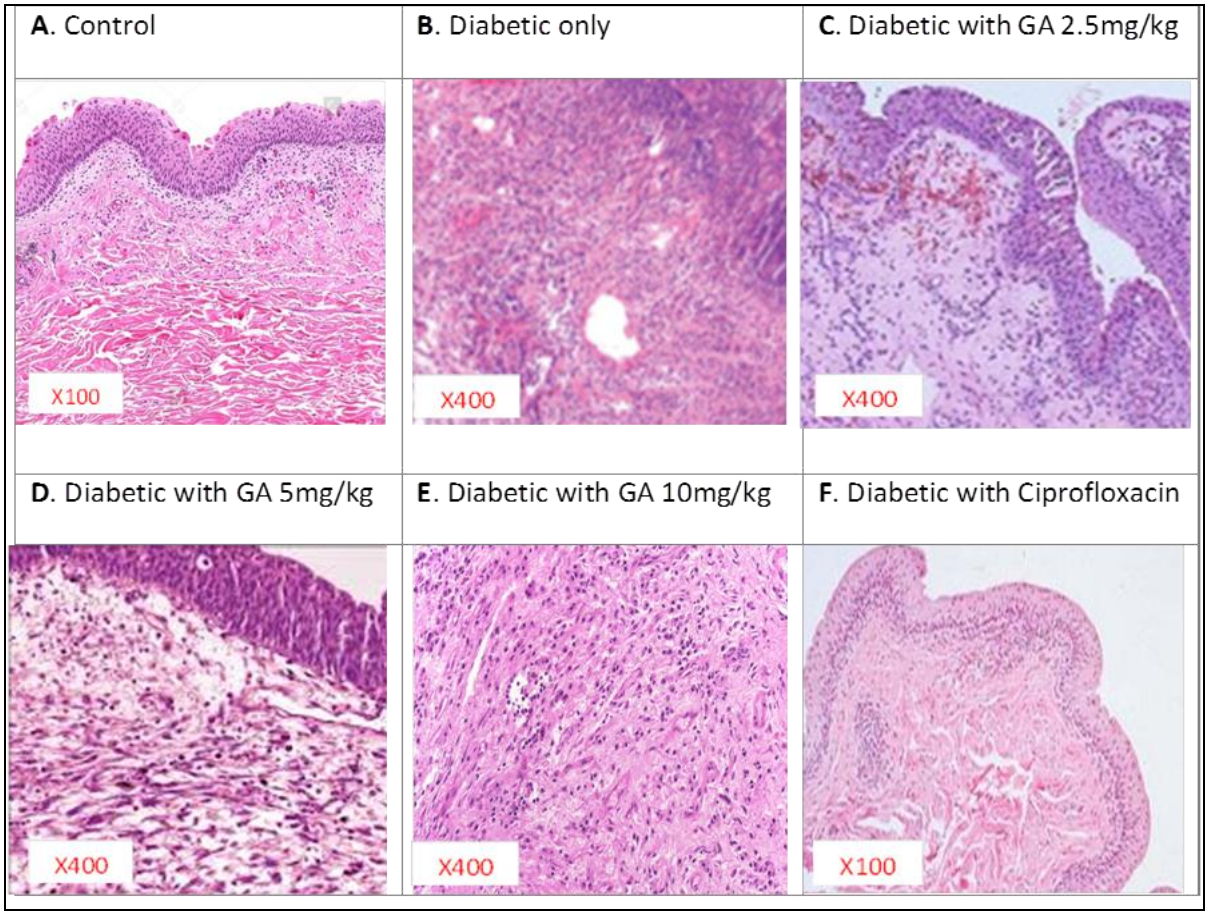

Fig. (1) Histopathological changes in urinary bladder of rats showing normal transitional epithelium and stroma in group A (Control). In group B (Diabetic); severe acute inflammation. Group B (Diabetic gallic acid treated); increasing dose of gallic acid (GA) decreased features of inflammation C, D and E. Mild inflammation in diabetic Ciprofloxacin treated group, F.

Discussion

The use of natural antioxidants in controlling hyperglycemia and prevention of oxidative damage are important strategies in preventing diabetes and its complications (23). The significant (P≤0.05) reduction in blood sugar levels by 30.7, 36.5 and 38.9% after administration of gallic acid at 2.5, 5 and 10 mg/kg respectively compared to diabetic rats reveals that gallic acid had improved the glycemic effect induced by alloxan. This hypoglycemic effect produced by gallic acid was demonstrated to be through stimulating the glucose uptake of cells by inducing GLUT4 translocation to the plasma membrane in Akt-independent manner and also by its anti-apoptotic and insulin secretagogue activity (24, 25). The reduction of severity of histopathological findings in gallic acid treated groups compared to diabetic group reveals an improvement in ameliorating the deleterious effects of hyperglycemia induced by alloxan (4, 12, 14). Concerning ciprofloxacin-treated diabetic rats, although the mean blood glucose levels was significantly (P≤0.05) higher than the controls however, the hyperglycemic state remained not significantly (P>0.05) different than those in the diabetic rats. This result may indicate that either ciprofloxacin did not had effect on hyperglycemic status in the rats or may have a role in maintaining the hyperglycemic status since ciprofloxacin found to produce hyperglycemia by blocking the closure of the insulinotropic effect of K/ATPase channel and also induces depletion of intracellular magnesium that can lead to insulin resistance in diabetic patients (26, 27). Furthermore, ciprofloxacin at recommended doses shown to induce oxidative stress and affect mitochondrial function (28) which most likely attributed to the maintenance of the hyper-glycemic state in the ciprofloxacin treated diabetic rats and coincide with the histopathological findings in bladder sections (27, 29). Fluoroquinolones are usually recommended for the treatment of many systemic infections in diabetic patients, therefore, their use in patients with diabetes should ideally be carefully watched (28, 29). The interest in antimicrobials derived from natural sources has increased in the last years due to the accepted safe status of these compounds (30). The antimicrobial activity of gallic acid found in this study is in accordance with others that found gallic acid is effective against *E.coli*, *P. auroginosa*, *P. mirabilis*, Salmonella spp**,** *S. aureus, M. tuberculosis, H. pylori* and candida (31, 32, 33). The mechanism proposed for the antimicrobial activities of gallic acid is irreversible changes in bacterial cell membrane by generating hydrogen peroxide leading to changes in cell surface hydrophobicity and charge, ultimately causing leakage of essential intracellular constituents' cytoplasmic content including calcium ions (34). Adhesion of *E.coli* to bladder epithelial cells was reduced by 31, 57 and 64% after administration of gallic acid at 2.5, 5 and 10 mg/kg respectively which could be related to its toxic activity against bacteria that resulted in decreased bacterial adhesion along with its ability in inhibiting the fimbriae or the adhesins that diffuses on cell surface of *E coli* which are necessary for the binding of *E. coli* to bladder epithelial cells thus inhibiting these mediators of urinary tract infections. These effects was proposed to be through the ability of gallic acid to inhibit bacterial growth, motility and bacterial-surface free energy of adhesion thus restricting the ability of *E.coli* to adhere to bladder cells (15). Moreover, gallic acid have been reported to interfere with bacterial quorum sensing, *i.e.*, the production of small signal molecules by bacterial cells of *E. coli* that trigger the exponential growth of a bacterial population thereby preventing adhesion and biofilm formation (15, 30, 35). The significant (P≤0.05) reduction of *E. coli* adhesion to bladder epithelial cells by ciprofloxacin is reported to be due its ability to induce oxidative changes in *E. coli*, which

ultimately cause distortion of plasma membrane and inhibit bacteria to initiate infection and inducing vacuolation of the internal components of the bacteria (36, 37). In conclusion; the hypoglycemic effect and inhibition of bacterial adhesion to bladder epithelial cells in this study through a light about necessity for designing further experimental investigations about the safety of therapeutic application of gallic acid and ciprofloxacin in improving diabetic status and for the treatment of lower urinary tract infection associated with diabetes.

References

1. Giacco, F. & Brownlee, M. (2010). Oxidative stress and diabetic complications. Cir. Res., 107 (9): 1058-1070.
2. Tiwari, B. K.; Pandey, K. B.; Abidi, A. B. & Rizvi, S. I. (2013). Markers of oxidative stress during diabetes mellitus. J. Biom., 1-8.
3. Fiorentino, T. V.; Prioletta, P.; Zuo, P. & Folli, F. (2013). Hyperglycemia- induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. Curr. Pharm. Des., 19 (32): 5695-5703.
4. Hanna-Mitchell, A. T.; Ruiz, G. W.; Daneshgari, F.; Liu, G.; Apodaca, G. & Birder, L. A. (2013). Impact of diabetes mellitus on bladder uroepithelial cells. Am. J. Physiol. Regul. Integr. Comp. Physiol., 304(2): 84-93.
5. Aswani, S. M.; Chandrashekar, U.; Shivashankara, K. & Pruthvi, B. (2014). Clinical profile of urinary tract infections in diabetics and non-diabetics. Australas Med. J., 7(1):29-34.
6. Funfstuck, R.; Nicolle, L. E.; Hanefeld, M. & Naber, K. G. (2012). Urinary tract infection in patients with diabetes mellitus. Clin. Nephrol., 77(1):40-48.
7. Boyko, E. J.; Fihn, S. D.; Scholes, D.; Abraham, L. & Monsey, B. (2005). Risk of urinary tract infection and asymptomatic bacteriuria among diabetic and nondiabetic postmenopausal women. Am. J. Epidemiol., 161(6):557-564.
8. Nitzan, O.; Elias, M.; Chazan, B. & Saliba, W. (2015). Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management. Diabetes Metab. Syndr. Obes., 8: 129-136.
9. Moorthy, S.; Keklak, J. & Klein, E. A. (2016). Perspective: Adhesion Mediated Signal Transduction in Bacterial Pathogens. Pathogens., 5(1): 23- 30.
10. Luthje, P. & Brauner, A. (2014). Virulence factors of uropathogenic E. coli and their interaction with the host. Adv. Microb. Physiol., 65:337-372.
11. Chen, J.; Mangelinckx, S.; Adams, A.; Wang, Z. T.; Li, W. L. & De Kimpe, N. (2015). Natural flavonoids as potential herbal medication for the treatment of diabetes mellitus and its complications. Nat. Prod. Commun., 10(1):187-200.
12. Punithavathi, V. R.; Prince, P. S.; Kumar, R. & Selvakumari, J. (2011). Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. Eur. J. Pharmacol., 650(1):465-471.
13. Booth, B. W.; Inskeep, B. D.; Shah, H.; Park, J. P.; Hay, E. J. & Burg, K. J. L. (2013). Tannic Acid Preferentially Targets Estrogen Receptor-Positive Breast Cancer. Int. J. Breast Cancer; 1-9.
14. Chao, J.; Huo, T. I.; Cheng, H. Y.; Tsai, J. C.; Liao, J. W.; Lee, M .S.; Qin, X. M.; Hsieh, M. T.; Mail, L. H. P. & Peng, W. H. (2014). Gallic acid ameliorated impaired glucose and lipid homeostasis in high fat diet-Induced NAFLD Mice. Plos One., 9 (6): e96969.

15. Borges, A.; Saavedra, M. J. & Simoes, M. (2012). The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. Biofouling, 28:755-767.
16. Lee, D. S. & Je, J. Y. J. (2013). Gallic acid-grafted-chitosan inhibits foodborne pathogens by a membrane damage mechanism. Agric. Food Chem., 61 (26): 6574-6579.
17. Maurya, H.; Mangal, V.; Gandhi, S.; Prabhu, K. & Ponnudurai, K. (2014). Prophylactic antioxidant potential of gallic Acid in murine model of sepsis. Int. J. Inflam., 1-9.
18. Nicolosi, D.; Tempera, G.; Genovese, C. & Furneri, P. M. (2014). Anti-Adhesion Activity of A2-type Proanthocyanidins (a Cranberry Major Component) on Uropathogenic E. coli and P. mirabilis Strains. Antibiotics, 3: 143-154.
19. King, A. J. F. (2012). The use of animal models in diabetes research. Br. J. Pharmacol., 166: 877-894.
20. Reyes, L.; Reinhard, M.; O'Donnell, L. J.; Stevens, J. & Brown, M. B. (2006). Rat Strains Differ in Susceptibility to *Ureaplasma parvum*-Induced Urinary Tract Infection and Struvite Stone Formation. Infect. Immun., 74 (12):6656-6664.
21. Clinical and Laboratory Standards Institute. (2010). Methods for antimicrobial dilution and disk susceptibility testing for infrequently-isolated or fastidious bacteria: approved guidelines. Publication no. M45-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
22. Geerlings, S. E.; Meiland, R.; Van Lith, E. C.; Brouwer, E. C.; Gaastra, W. & Hoepelman, A. I. (2002). Adherence of type 1-fimbriated Escherichia coli to uroepithelial cells: more in diabetic women than in control subjects. Diabetes Care, 25(8):1405-1409.
23. Umadevi, S.; Gopi, V.; Parthasarathy, A. & Elangovan, V. (2011). Ameliorative potential of gallic acid on the activation of ROS and down-regulation of antioxidant enzymes in cardiac tissue of rats infused with advanced glycation end products. J. Appl. Pharm. Sci., 1 (7): 189-193.
24. Prasad, C. N.; Anjana, T.; Banerji, A. & Gopalakrishnapillai, A. (2010). Gallic acid induces GLUT4 translocation and glucose uptake activity in 3T3-L1 cells. FEBS Letters, 584: 531-536.
25. Sameer Mahmood, Z.; Raji, L.; Saravanan, T.; Vaidya, A.; Mohan, V. & Balasubramanyam, M. (2010). Gallic acid protects RINm5F beta-cells from glucolipotoxicity by its antiapoptotic and insulin-secretagogue actions. Phytother. Res., 24(4):82-94.
26. Ghaly, H.; Kriete, C.; Sahin, S.; Pfloger, A.; Holzgrabe, U.; Zunkler, B. J. & Rustenbeck, I. (2009). The insulinotropic effect of fluoroquinolones. Biochem. Pharmacol., 77(6):1040-1052.
27. Telfer, S. J. (2014). Fluoroquinolone antibiotics and type 2 diabetes mellitus. Med. Hypotheses, 83: 263-269.
28. Talla, V. & Veerareddy, P. R. (2011). Oxidative Stress Induced by Fluoroquinolones on Treatment for Complicated Urinary Tract Infections in Indian Patients. J. Young Pharm., 3(4): 304-309.
29. Ghaly, H.; Jorns, A. & Rustenbeck, I. (2014). Effect of fluoroquinolones on mitochondrial function in pancreatic beta cells. Eur. J. Pharm. Sci., 52:206-214.

30. Samy, R. P. & Gopalakrishnakone, P. (2010). Therapeutic Potential of Plants as Anti-microbials for Drug Discovery. Evid Based Complement Alternat Med., 7(3): 283-294.
31. Al-Zahrani, S. H. M. (2012). Antibacterial activities of gallic acid and gallic acid methyl ester on methicillin-resistant *Staphylococcus aureus.* J. Am. Sci., 8(2): 7-12.
32. Diaz-Gomez, R.; Lopez-Solis, R.; Obreque-Slier, E. & Toledo-Araya, H. (2013). Comparative antibacterial effect of gallic acid and catechin against *Helicobacter pylori.* LWT - Food Sci. Technol., 54: 331-335.
33. Teodoro, G. R.; Ellepola, K.; Seneviratne, C. J. & Koga-Ito, C. Y. (2015). Potential Use of Phenolic Acids as Anti-Candida Agents: A Review. Front Microbiol., 6:1420-1431.
34. Borges, A; Ferreira, C.; Saavedra, M. J. & Simoes, M. (2013). Antibacterial activity and mode of action of ferulic and Gallic acids against Pathogenic Bacteria. Microbial Drug Res., 19 (4):256-265.
35. Kang, M. S.; Oh, J. S.; Kang, I. C.; Hong, S. J. & Choi, C. H. (2008). Inhibitory effect of methyl gallate and gallic acid on oral bacteria. J. Microbiol., 46 (6):744-750.
36. Mohamed, A. M. & Mohammed Ali, N. A. K. (2012). The Effect of Fluoroquinolone Antibacterials on the Adhesion of *E.coli* 078 to Buccal Epithelial cells. J. Adv. Biom. Pathobiol. Res., 2 (4): 129-136.
37. Jensen, P. O.; Briales, A.; Brochmann, R. P.; Wang, H.; Kragh, K. N.; Kolpen, M.; Hempel, C.; Bjarnsholt, T.; Hoiby, N. & Ciofu, O. (2014). Formation of hydroxyl radicals contributes to the bactericidal activity of ciprofloxacin against Pseudomonas aeruginosa biofilms. Pathog. Dis., 70(3):440-443.