Original Article

Evaluation of preservative effectiveness of gallic acid derivatives in aluminum hydroxide gel-USP

Abstract

Background: Preservatives are added to most of the pharmaceutical preparations to prevent them from deterioration throughout their shelf life. Literature reveals that the common synthetic preservatives have many limitations, such as development of microbial resistance (in due course of time) and several serious side-effects. **Aim:** The aim of this study is to find out new preservatives synthesized from natural sources, which may have better efficiency than the existing synthetic preservatives. The derivatives of naturally occurring gallic acid were subjected for their preservative efficacy study. Their preservative efficiency was evaluated and compared with the standard parabens. **Materials and Methods:** The selected amide, anilide and ester derivatives of gallic acid were subjected to preservative efficacy testing in an official antacid preparation, {aluminum hydroxide gel-USP (United States Pharmacopoeia)} against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Candida albicans,* and *Aspergillus niger* as representative challenging microorganisms as per USP 2004 guidelines. **Results:** The selected derivatives were found to be effective against all selected strains and showed preservative efficacy comparable to that of standard and even better in case *E. coli, C. albicans* and *A. niger.* The 8-hydroxy quinoline ester derivative showed better preservative efficacy than standard as well as other derivatives. **Conclusion:** The newly synthesized gallic acid preservatives were found to be effective in the proposed pharmaceutical preparation (Aluminium Hydroxide Gel – USP). Also, the synthesized preservatives have shown comparative and even better efficacy than the existing parabens and hence they have potential for use in pharmaceutical preparations.

Key words:
Amides, esters, gallic acid, log CFU/ml (Colony forming unit per ml), preservative

Introduction

Antimicrobial preservatives are added to multi-dose pharmaceutical products for the purpose of inhibiting and killing the growth of microorganisms which may be introduced during the multiple withdrawals of the product from their containers.[1] Deterioration of pharmaceutical preparations due to growth of microorganisms has become a challenge and needs preservation to maintain their shelf life.[2]

Moreover, the commonly used synthetic preservatives have shown very serious side-effects viz. the benzalkonium chloride was reported to be genotoxic and cytotoxic by the study of Deutschle *et al*., 2006 and further Graf *et al*., 2001 reported that it may also cause mucosal damage.[3,4] Liao *et al*., 2011 reported about thiomerosal commonly used in ocular and nasal preparations as cytotoxic.[5] The use of parabens may cause skin cancer, genotoxicity, and breast cancer as reported by the study of Darbre *et al*., 2008. Furthermore, the microorganisms commonly develop resistance to antimicrobials and in some cases are able to degrade many commonly used preservatives especially *p*-hydroxybenzoates, e.g., paraben.[6,7]

The United States and British Pharmacopoeias describe official methods for evaluation of preservative system.[8,9] Preservative efficacy test (challenge test) involves the artificial introduction of representative

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microorganisms including gram positive and gram negative bacteria, mold and yeast into the product under study, in sufficient amounts followed by the collection of kinetic information regarding the loss of their viability. The simple organic acids have been reported in the literature as potential preservative such as the capryllic acid, veratric acid, 2,4 hexadienoic acid, and anacardic acid.[10-13] Furthermore, the gallic acid and its derivatives possess a wide spectrum of biological activities such as antimicrobial, anticancer, antiviral, anti-inflammatory, analgesic, and anti-HIV activities.[14-19]

In view of the potential of microorganisms developing resistance to most common preservatives, it became imperative to develop newer and stronger preservatives. Further, in view of the reported toxicity potential of common synthetic preservatives, it would be quite judicious to develop the preservatives based on the natural sources such as gallic acid. In this context, amide and ester derivatives of gallic acid were investigated for preservative efficacy in the present work. The preservative efficacy of most effective amide, anilide and ester derivatives of gallic acid against gram positive *Staphylococcus aureus MTCC* 2901 {The Microbial Type Culture Collection and Gene Bank (MTCC)}, *Bacillus subtilis MTCC* 2063, gram negative *Escherichia coli MTCC* 1652, fungal strains *Aspergillus niger MTCC* 8189 and *Candida albicans MTCC* 227 was investigated and compared them with the standard preservatives methyl and propyl paraben, in aluminum hydroxide gel-USP.[20]

Materials and Methods

Materials

Nutrient agar, nutrient broth, sabouraud dextrose agar and sabouraud dextrose broth were obtained from Himedia, Mumbai. Mannitol, methyl and propyl paraben were obtained from CDH, Mumbai.

Methods

Aluminum hydroxide gel-USP was used as the pharmaceutical product for evaluation of preservative efficacy testing.

Formula for preparation of aluminum hydroxide gel-USP 2004

Aluminum hydroxide gel, 36 g; Mannitol, 7 g; Methyl paraben, 0.2 g; propyl paraben, 0.02 g; saccharin, 0.05 g; peppermint oil, 0.005 ml; alcohol, 1 ml; purified water q.s., 100 ml.

The weighed quantity of aluminum hydroxide gel and mannitol were triturated with 50 ml of water in a mortar. Methyl paraben, propyl paraben, saccharin, and peppermint oil were dissolved in alcohol and added to above mixture and triturated well. The volume was made up to 100 ml with purified water followed by its sterilization by autoclaving.

For preservative efficacy testing, the aluminum hydroxide gel was prepared using the preservatives mentioned in Table 1 by replacing methyl paraben and propyl paraben from the above formula. The equimolar amount of selected preservatives [Figure 1] were calculated with reference to the amount of methyl paraben (0.0013 mol) and added into aluminum hydroxide gel.[21]

Table 1: Amount of selected preservatives added in aluminum hydroxide gel-USP

Preservative	Amount (g)
Gallic-8-hydroxy quinoline ester	0.386
Gallic 2-methyl 5-nitro anilide	0.395
Gallic N, N-dimethyl amide	0.256
Gallic naphthyl amide	0.383

USP – United States Pharmacopoeia; CFU – Colony Forming Units

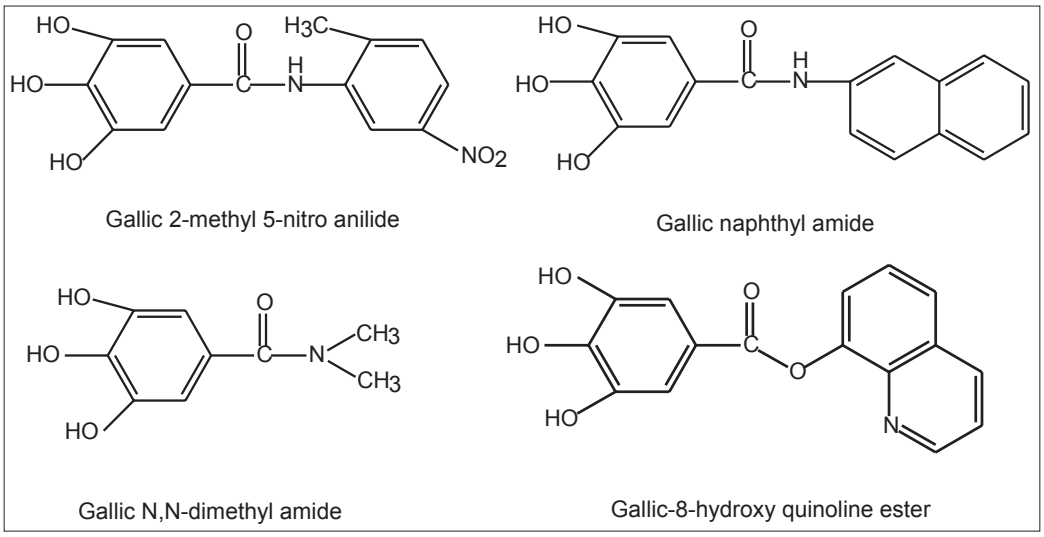

Figure 1: Structures of selected gallic acid derivatives

Strains

S. aureus MTCC 2901, *B. subtilis MTCC* 2063, *E. coli MTCC* 1652, *C. albicans MTCC* 227 and *A. niger MTCC* 8189 were used in this study were common contaminants and prescribed in USP for preservative efficacy testing in the pharmaceutical preparations.

Preservative efficacy testing in aluminum hydroxide gel-USP 2004

The preservative efficacy test was performed essentially following the standard protocol described in USP-2004. In all cases, the preservative efficacy test was done in aluminum hydroxide gel-USP with and without the preservative system. The unpreserved product was used as a control to evaluate the viability of the inoculated cells and their ability to grow in the product.

Preparation of inoculum

The representative microorganisms were inoculated in nutrient agar I.P. (Indian Pharmacopoeia) (*S. aureus, B. subtilis, E. coli*) and sabouraud agar I.P. (*C. albicans, A. niger*). The seeded plates were incubated at 37°C for 24 h (*S. aureus, B. subtilis, E. coli*), 37°C for 48 h (*C. albicans*) and 25°C for 7 d (*A. niger*). After the incubation period, suspensions of microorganisms were prepared in sterile saline solution (0.9% w/v NaCl) to give a microbial count of 1×10^4 CFU/ml.[11]

Test procedure

Aluminum hydroxide gel-USP in their final container was used in the challenge test. The preparation was inoculated with the microbial cell suspension with a cell count of 1×10^4 CFU/ml. The inoculum never exceeded 1% of the volume of the product sample. Inoculated samples were mixed thoroughly to ensure homogeneous microorganism distribution and incubated. The CFU/ml of the product was determined at an interval of 0, 7, 14, 21, and 28 days on an agar plate. The log values of the number of CFU/ml [Tables 2-6] of aluminum hydroxide gel was calculated and compared as per the guidelines of USP 2004.

Criteria of acceptance for preservative system

As per USP 2004 requirement for antacid made with an aqueous base, preservative effectiveness is met if there is no increase from initial calculated count at 14th and 28th days in case of bacteria, yeast and molds and where, no increase is defined as not more than 0.5 $\log_{10}$ higher than the previous value measured.[9]

Results and Discussion

The results of preservative efficacy testing performed in triplicate were reported as mean values in Tables 2-6. According to the values of Table 2, the amide, anilide and ester derivatives of gallic acid have values of log CFU/ml within the prescribed limit and the results were comparable to that of the standard preservative against *B. subtilis*.

Table 2: Bacterial count of *Bacillus subtilis* in aluminum hydroxide gel-USP supplemented with preservatives

Preservative added	Log CFU/ml (time in days)				
	0	7	14	21	28
Gallic-8-hydroxy quinoline ester	0.467	0.699	0.681	0.873	0.719
Gallic 2-methyl 5-nitro anilide	0.929	0.921	0.903	0.748	0.763
Gallic N, N-dimethyl amide	0.628	1.155	1.125	2.049	0.865
Gallic naphthyl amide	0.531	0.456	0.488	0.572	0.740
Standard	0.602	0.301	0.000	0.301	0.477
Control	0.903	0.477	0.602	0.778	0.845

USP – United States Pharmacopoeia; CFU – Colony Forming Units

Table 3: Bacterial count of *Staphylococcus aureus* in aluminum hydroxide gel-USP supplemented with preservatives

Preservative added	Log CFU/ml (time in days)				
	0	7	14	21	28
Gallic-8-hydroxy quinoline ester	0.523	0.903	0.748	0.623	0.668
Gallic 2-methyl 5-nitro anilide	0.721	0.681	0.819	0.845	1.089
Gallic N, N-dimethyl amide	0.377	0.380	0.972	0.398	0.653
Gallic naphthyl amide	0.301	0.535	0.651	0.477	0.829
Standard	0.523	0.903	0.748	0.623	0.668
Control	0.721	0.681	0.819	0.845	1.089

USP – United States Pharmacopoeia; CFU – Colony Forming Units

Table 4: Bacterial count of *Escherichia coli* in aluminum hydroxide gel-USP supplemented with preservatives

Preservative added	Log CFU/ml (time in days)				
	0	7	14	21	28
Gallic-8-hydroxy quinoline ester	0.824	0.434	0.574	0.653	0.602
Gallic 2-methyl 5-nitro anilide	0.699	0.677	1.000	0.954	1.000
Gallic N, N-dimethyl amide	0.959	0.434	0.602	0.653	0.602
Gallic naphthyl amide	0.721	0.436	1.079	0.954	1.000
Standard	0.778	0.000	0.602	0.302	0.698
Control	0.845	0.602	0.778	0.954	1.041

USP – United States Pharmacopoeia; CFU – Colony Forming Units

Table 5: Fungal count of *Candida albicans* in aluminum hydroxide Gel-USP supplemented with preservatives

Preservative added	Log CFU/ml (time in days)				
	0	7	14	21	28
Gallic-8-hydroxy quinoline ester	1.000	1.273	1.342	0.824	0.796
Gallic 2-methyl 5-nitro anilide	0.724	0.413	0.942	0.347	0.398
Gallic N, N-dimethyl amide	0.954	0.699	0.719	0.824	0.921
Gallic naphthyl amide	1.255	0.921	0.865	0.959	1.030
Standard	0.301	0.698	0.602	0.778	0.000
Control	0.477	0.778	0.845	0.845	0.903

USP – United States Pharmacopoeia; CFU – Colony Forming Units

Table 6: Fungal count of *Aspergillus niger* in aluminum hydroxide gel-USP supplemented with preservatives

Preservative added	Log CFU/ml (time in days)				
	0	7	14	21	28
Gallic-8-hydroxy quinoline ester	0.699	0.778	1.177	0.653	0.564
Gallic 2-methyl 5-nitro anilide	0.370	0.556	1.473	0.875	0.196
Gallic N, *N*-dimethyl amide	1.398	1.352	0.679	1.000	1.740
Gallic naphthyl amide	0.796	0.954	0.820	0.954	0.564
Standard	0.301	0.301	0.698	0.000	0.477
Control	0.698	1.079	0.954	1.000	1.079

USP – United States Pharmacopoeia; CFU – Colony Forming Units

Gallic N, N-dimethyl amide derivative shown a change of more than 0.5 log value of CFU/ml on 14 day and was considered to be less effective preservative against *S. aureus* while all other amide, anilide and ester derivatives of gallic acid were found to be effective preservative and the results were also comparable to that of the standard [Table 3].

The naphthyl amide derivative of gallic acid have shown an increase of more than 0.5 log CFU/ml on 14 day and was assumed to be less effective as compared to other derivatives. Furthermore, the standard was found to be less effective on 14 day as there was considerable change in the values of log CFU/ml and hence the 8-hydroxy ester, 2-methyl-5-nitro anilide and N, N-dimethyl amide derivatives of gallic acid were even more active preservative than the standard against *E. coli* [Table 4].

2-methyl-5-nitro anilide derivative of gallic acid have shown increment of more than the prescribed limit for log CFU/ml on 14 day and hence was less effective as compared to other derivatives. Furthermore, the standard have shown a change of more than 0.5 log value on 28 day and hence was assumed to be less active preservative against *C. albicans*. The 8-hydroxy ester, naphthyl amide and *N, N*-dimethyl amide derivatives of gallic acid were within the prescribed limits of challenge test on 14 and 28 days [Table 5].

2-methyl-5-nitro anilide and N, N-dimethyl amide derivatives of gallic acid have shown a significant change in log CFU/ml values on 14 and 28 day, which is more than the criteria prescribed for challenge test and hence were considered to be less active preservative against *A. niger*. Even, in case of standard the change on 28 day was just near to 0.5 log value and was considered as less active preservative against *A. niger*. The other derivatives of gallic acid were within the prescribed limit of preservative efficacy testing criteria on 14 and 28 day [Table 6].

Conclusion

The study has shown the preservative potential of 8-hydroxy quinoline ester, naphthyl amide and N, N-dimethyl amide and 2-methyl-5-nitro anilide derivatives of gallic acid in pharmaceutical preparation. The selected amide, anilide and ester derivatives of gallic acid were found effective against all selected strains and showed preservative efficacy comparable to that of standard and even better in case of *E. coli*, *C. albicans* and *A. niger*. The 8-hydroxy quinoline ester derivative showed better preservative efficacy than standard as well as other derivatives and it can be a better alternative to the existing preservatives for use in the pharmaceutical preparations.

References

1. Moser CL, Meyer BK. Comparison of compendial antimicrobial effectiveness tests: A review. AAPS PharmSciTech 2011;12:222-6.
2. Khatkar A, Nanda A, Narasimhan B. Preservatives-associated problems and possible alternatives. In: Tiwari SK, Singh B, editors. Current Trends in Biotechnology. Germany: Lambert Academic Publisher; 2012. p. 100-20.
3. Deutschle T, Porkert U, Reiter R, Keck T, Riechelmann H. *In vitro* genotoxicity and cytotoxicity of benzalkonium chloride. Toxicol *in vitro* 2006;20:1472-7.
4. Graf P. Benzalkonium chloride as a preservative in nasal solutions: Re-examining the data. Respir Med 2001;95:728-33.
5. Liao WC, Chou CT, Kuo CC, Pan CC, Kuo DH, Shieh P, *et al*. Effect of thimerosal on Ca movement and apoptosis in PC3 prostate cancer cells. Drug Dev Res 2011;72:330-6.
6. Darbre PD, Harvey PW. Paraben esters: Review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. J Appl Toxicol 2008;28:561-78.
7. Close JA, Neilsen PA. Resistance of a strain of *Pseudomonas cepacia* to esters of p-hydroxybenzoic acid. Appl Environ Microbiol 1976; 31:718-22.
8. British Pharmacopoeia. Preservative and Antimicrobial. Appendix XVI C, London: British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare products Regulatory Agency; 1993. p. A191-2.
9. The United States Pharmacopoeia. Antimicrobial effectiveness testing. Rockville: United States Pharmacopoeial Convention Inc.; 2004. p. 2148-50.
10. Chaudhary J, Rajpal AK, Judge V, Narang R, Narasimhan B. Preservative Evaluation of caprylic acid derivatives in aluminium hydroxide gel – USP. Scientia Pharmaceutica 2008;76:533-99.
11. Ohlan S, Ohlan R, Narasimhan B, Judge V, Narang R. Evaluation of veratric acid derivatives as preservative in aluminium hydroxide gel. Acta Pharmaceutica Sciencia 2008;50:241-5.
12. Judge V, Narasimhan B, Narang R, Ohlan R, Ohlan S. Preservative evaluation of novel 2,4-hexadienoic acid derivatives in aluminium hydroxide gel – USP. Scientia Pharmaceutica 2008;76:269-77.
13. Narasimhan B, Singh N, Panghal A, Dhake A. Efficiency of anacardic acid as preservative in tomato products. J Food Process Preserv 2008;32:600-9.
14. Chanwitheesuk A, Teerawutgulrag A, Kilburn JD, Rakariyatham N. Antimicrobial gallic acid from *Caesalpinia mimosoides Lamk*. Food Chem 2007;100:1044-8.
15. Saxena HO, Faridi U, Srivastava S, Kumar JK, Darokar MP, Luqman S, *et al*. Gallic acid-based indanone derivatives as anticancer agents. Bioorg Med Chem Lett 2008;18:3914-8.
16. Thapa M, Kim Y, Desper J, Chang KO, Hua DH. Synthesis and antiviral activity of substituted quercetins. Bioorg Med Chem Lett 2012;22:353-6.
17. Arunkumar S, Ilango K, Manikandan RS, Manikandan N. Synthesis and anti-inflammatory activity of some novel pyrazole derivatives of gallic acid. E-J Chem 2009;6:S123-8.
18. Krogh R, Yunes RA, Andricopulo AD. Structure-activity relationships for the analgesic activity of gallic acid derivatives. Farmaco 2000;55:730-5.
19. Kratz JM, Andrighetti-Fröhner CR, Kolling DJ, Leal PC, Cirne-Santos CC, Yunes RA, *et al*. Anti-HSV-1 and anti-HIV-1 activity of gallic acid and pentyl

gallate. Mem Inst Oswaldo Cruz 2008;103:437-42.

20. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. Bombay: Vargheese Publishing House; 1987. p. 498-9.
21. Narang R, Narasimhan B, Judge V, Ohlan S, Ohlan R. Evaluation of preservative effectiveness in an official antacid preparation. Acta Pharmaceutica Sciencia 2009;51:225-9.

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