Proceedings of

ICMR Sponsored National Conference on

Challenging Issues and Technological Approaches in Medicine

CITAM 2016

Editors

Dr.S.Anantha Lakshmi
Dr.R.Pappa Ammal
Dr.Kala Chandrasekaran
Mrs.S.Bhuvaneswari
Mrs.N.Kalpana

Published by

Department of Science and Humanities
Faculty of Engineering
Avinashilingam Institute for Home Science and Higher Education for Women
Coimbatore - 641 108

Challenging Issues and Technological Approaches in Medicine

CITAM 2016

Vijaya Pathippagam

20 Raja Street, Coimbatore - 1

(0422) 2385614/2382614

www.vijayapathippagam.com

ISBN : 81-8446-755-9

2016

Published by

Department of Science and Humanities

Faculty of Engineering

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore - 641 108

Disclaimer : Editors are not responsible for the scientific authentication or the originality of the contents published in this compilation.

Printed at

Aruna Enterprises, Chennai - 1

Cover page designed by **S. Shobiya** B.E IT (2013-2017)

Faculty of Engineering , Avinashilingam University

Pages : xxiv + 204

Price : Rs.650/-

Glycyrrhiza glabra – a Natural Panacea against Diarrhoea and Cancer

[1]M.Ramya, [2]Dr.M.Sylvia Suba priya & [3]Dr.Nalini.B

[1]Research Scholar, [2]Associate Professor & [3]Assistant Professor
[1,2]Department of Food Science & Nutrition, [3]Department of Physics,
Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore
[1]ramvindrheya@gmail.com, [2]sylviadevaprasad@yahoo.co.in [3]jyothsnalalin99@gmail.com

ABSTRACT

The major food borne outbreaks of diarrhoeal diseases are caused by microbes such as *Escherichia coli, Salmonella enterica* and *Shigella dysenteriae*. The present study was conducted to develop nanoparticle coatings from the medicinal plant, *Glycyrrhiza glabra* against diarrhea and carcinoma. The GC/MS of nanoparticles reveals the following active phyto constituents namely 9-octadecenal, tetradecanoic acid, oleic Acid, z, z 2, 5-Pentadecadien-1-ol, cis-9,10-epoxyoctadecan-1-ol and n-hexadecanoic acid. The antioxidant and thin layer chromatography of *Glycyrrhiza glabra* nanoparticles showed a maximum of 520 µg and phenolic compounds. The antimicrobial activity were tested by disc diffusion showed microbial inhibition of 11, 15 and 14 mm against *E.coli* (MTCC 40), *S.enterica* (MTCC 3219) and *S.dysenteriae* (PSGIMS&R) respectively. Therefore, commercially available food packages such as PET bottles, zip lock covers and infant feeding bottles were coated with nanoparticles and tested for shelf-life for ten days. In nanocoated PET bottles and ziplock covers stored with tomato puree showed that till 6th day, there was no microbial growth. On the 10th day, 57, 68 and 88 percentage of microbial inhibition was observed in PET bottles against *E.coli* (MTCC 40), *S.enterica* (MTCC 3219) and *S.dysenteriae* (PSGIMS&R) respectively. In nanocoated zip lock covers showed 38, 63 and 83 percentage of microbial inhibition was observed against diarrhoeal pathogens. Nanocoated feeding bottles, showed a maximum microbial inhibition (84percentage) against diarrhoeal pathogens. Gel electrophoresis showed DNA damage to the Hela and Liver carcinoma cell lines and 50 and 48 per cent apoptosis in the two cell lines respectively. Therefore, *Glycyrrhiza glabra* nanoparticles are effective in diarrrhoea prevention and cancer treatment.

Introduction

BPA is a carcinogen and it gets deposited to human body causing the breast and prostate cancer. Moreover, exposure to BPA increases the possibility of brain tumour or meningioma. BPA is a thyroid-disrupting chemical, which also affect pregnant women, neonates and small children. BPA is also associated with increasing neurobehavioral problems, increasing prevalence of obesity and type2 diabetes and affecting the immune system effects (Ghosh *et al.*, 2015).

Diarrhoea is the third leading cause of childhood mortality in India accounting for 13% of all deaths/year in children under 5 years of age (Bhan *et al.*, 2013). Diarrheal disease is an important public health problem among under-five children in developing countries (Bassani *et al.*, 2010). Total diarrheal deaths in India among children aged 0-6 years was estimated to be 158,209 and the mortality due to diarrhea was 9.1%. The average estimated incidence of diarrhea in children aged 0-6 years was 1.71 and 1.09 episodes/person/year in rural and urban areas (Bassani *et al.*, 2010). According to National Family Health Survey-3 (NFHS-3) report, 9% of all under-five children were reported to be suffering from diarrhea in last 2 weeks. Hence, this study investigated the effectiveness of active compounds from the extracts obtained from *Glycyrrhiza glabra* chelated with silver nanoparticles. These particles induces apoptosis in cancer reduces the diarrheal causing pathogens in nanocoated plastics. Thus, the objectives of this study were to demonstrate the silver nanoparticles and their synergistic use with extracts from *Glycyrrhiza glabra* for medicinal use.

2. Materials & Methods

2.1. Identification of *Glycyrrhiza glabra* constituents using Gas Chromatography (GC)

The GC analysis was performed using a SHIMADZU QP 5050A, GC/MS-5989Binstrument. The GC was fitted with a DBI (30 m × 0.53 mm × 1.5 µm) fused silica capillary column. The GC carrier was helium (flow rate 1ml/min) at an Ionization mode of EL 70eV. Temperature program was 40°C (static for 2 min), then it increased to 160°C at a rate of 2°C/min.

2.2. Identification of compounds by Thin layer chromatography

To determine active compounds in the plant extract was carried out using thin layer chromatography. The silver nanoparticles was spotted on TLC sheet coated with thin layer of Silica gel (Macherey-Nagel, Germany) and it was kept inside the chamber containing solvent (N- Butanol, Acetic Acid and Distilled water - 8:2:2 v/v) and allowed to develop chromatogram.

2.3. To determine Antioxidant activity of synthesized Silver Nanoparticle (AgNPs) by FERRIC REDUCINGANTIOXIDANT POWERASSAY (FRAP):-

FRAP assay was performed by taking a known volume of the sample was made up to 3 ml with phosphate buffer, 1% potassium ferricyanide and incubate in water bath for 20 minutes at 500 °C. Allowed to cool and then added 10% TCA, 2.5 ml distilled water and 0.5 ml ferric chloride, kept for 10 minutes in room temperature and the absorbance was read at 700 nm against standard ascorbic acid (Benzie and Strain, 1999).

2.4. MTT Assay

Hela and Liver carcinoma cell lines were seeded into a 96-well plate and incubated 24 hrsovernight at 37 °C to ensure cell adhesion and confluence in the wells. The medium was replaced with a fresh one containing silver nanoparticles and extracts from the plant in different concentrations (20-200 µl) for 24 hr. The effects of silver nanoparticles on the cells viability were estimated by MTT assay using 3-4,5-dimethylthiazol-2yl),2,5-diphenyl-2H-tetrazolium bromide (MTT).

2.5. DNA Fragmentation Assay (DNA)

DNA fragmentation has long been used to distinguish apoptosis from necrosis, as the most reliable method for detection of apoptotic cells. Hela and Liver carcinoma cells were plated in 16 six-well tissue culture plates and incubated at 37 °C overnight. These cells were treated with 2.0 mL of stock solution suspended in Dulbecco's modified eagle's medium (DMEM) containing 10% Fetal Bovine Serum. The cells were then, treated with 2 µg/mL Doxorubicin hydrochloride used as controls, along with the silver nanoparticle and extracts.

2.6. Antibacterial testing of silver nanoparticles (*Glycyrrhiza glabra*)

The silver nanoparticles were tested against the dirrhoeal causing pathogens by disc diffusion test. To the agar, 102 cfu/ml amount of each bacterial culture were spreaded to the petrid plate and were tested for the effectiveness of silver nanoparticles at varied concentrations along with the control. Then, the petrid plates were placed in an incubator aerobically at 37°C for 24 hours.

2.7. Coating (*Glycyrrhiza glabra*) nanoparticles onto Commercially available food packages:

To the commercially available food packages namely PET bottles, Ziplock covers and infant feeding bottles. Around 30 µl of silver nanoparticle were coated on to the surface of the plastic material and were tested against the diarrhoeal pathogens such as *E.coli* (MTCC 40), *S.etnerica* (MTCC 3219) and *S.dysenteriae* (PSGIMS&R) and were tested for its shelf-life for a period of ten days.

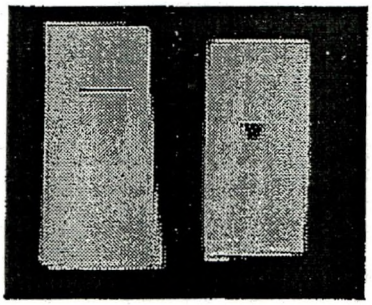
3.Results and Discussion:

3.1. Gas chromatography

There are six major phytochemical compounds were identified in *Glycyrrhiza glabra* nanoparticles such as 9-Octadecenal, Z,Z-2,5-Pentadecadien-1-ol, Tetradecanoic acid, n-Hexadecanoic acid , Oleic Acid and cis-9,10-Epoxyoctadecan-1-ol were found to possess antimicrobial, antioxidant and anticancer property. Further, these phytochemicals acts as a natural reducing agent in the biosynthesis of nanoparticles from plant extract

3.2. Thin Layer Chromatography :

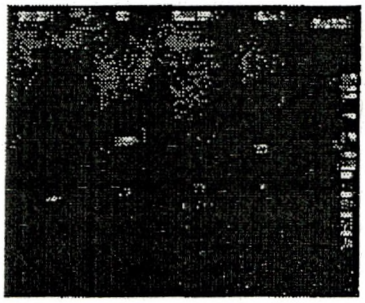
Figure 1 - Thin Layer chromatography of *Glycyrrhiza glabra*



From the figure 1 depicts that the silver nanoparticles of *Glycyrrhiza glabra* showed the presence of phenolic compounds as a purple colour. These constituents possess the antioxidant and antimicrobial property which could be as a nanocoating in commercially available food packages such as PET bottles, ziplock covers and Infant feeding bottles

3.3. MTT Assay :

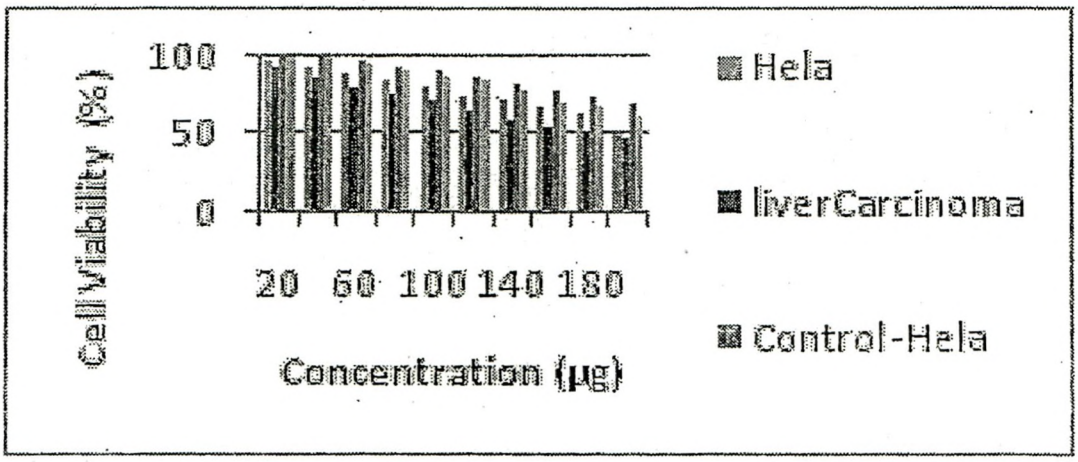
Figure 2 - MTT Assay of synthesized silver nanoparticles (*Glycyrrhiza glabra*) against hela and liver cancer



From the figure 5 showed that the cell viability determination was made using MTT assay which was studied for *Glycyrrhiza glabra* causing a significant cytotoxicity. From the analysis, it was found that 200 µl of silver nanoparticles showed 50 percent apoptosis in hela cell line and 48 percent in human carcinoma apoptosis at a concentration of 200µl compared to control. Thus, we hypothesize that apoptosis of cancer cells could be the possible mechanism induced by the cytotoxic effect of biosynthesized silver nanoparticles from *Glycyrrhiza glabra*.Despite the widespread use of silver nanoparticles (*Glycyrrhiza glabra*) there are only few research work in the context of apoptosis to determine its cytotoxicity.

3.4. Gel Electrophoresis

Figure 3 -Gel Electrophoresis of Hela and Liver carcinoma cells



The silver nanoparticles (*Glycyrrhiza glabra*) has led to a longer tail DNA damage with Hela and Liver carcinoma cell lines compared with control and marker. However, Figure 4 showed that DNA gel electrophoresis of DNA fragmentation was enhanced with increasing exposure to silver nanoparticles (*Glycyrrhiza glabra*).

3.5. Shlef-life Study of Nanocaoted food packages :

The nanocoated food packages such as PET bottles, Ziplock covers and infant feeding bottles were tested for its antidiarrhoeal efficacy and its shelf-life for a period of ten days.

Table 1- Shelf-life study of tomato puree in nanocoated PET bottles

Days of	E.oli (MTCC 40)				S.enterica (MTCC 3219)				S.dysenteriae (PSGIMSR)			
Testing	E1	E2	C	%	E1	E2	C	%	E1	E2	C	%
0th day	NIL	NIL	17	NIL	NIL	NIL	12	NIL	NIL	NIL	39	NIL
2nd day	NIL	NIL	26	NIL	NIL	NIL	28	NIL	NIL	NIL	65	NIL
4th day	NIL	NIL	53	NIL	NIL	NIL	74	NIL	NIL	NIL	197	NIL
6th day	NIL	NIL	81	NIL	NIL	NIL	118	NIL	NIL	NIL	243	NIL
8th day	46	50	113	57.52	89	93	162	43.83	24	27	TNTC	91.5
10th day	128	131	TNTC	56.83	96	98	TNTC	67.67	38	36	TNTC	87.7

From table I showed that the shelf-life study of tomato puree in nanocoated PET bottles showed that on 8th day, the percentage of microbial inhibition was found to be 58, 44 and 92 percent against *E.coli*(MTCC 40), *S.enterica*(MTCC 3219) and *S.dysenteriae* (PSGIMS&R) respectively. On 10th day, among the three diarrrhoeal species, a maximum microbial inhibition of 88 percent was observed against *S.dysenteriae* compared to the control.

Table 2- Shelf-life study of tomatopuree in nanocoated ziplock covers

Days of	*E.oli*(MTCC 40)				*S.enterica*(MTCC 3219)				*S.dysenteriae*(PSGIMSR)			
Testing	E1	E2	C	%	E1	E2	C	%	E1	E2	C	%
0th day	NIL	NIL	11	NIL	NIL	NIL	7	NIL	NIL	NIL	43	NIL
2nd day	NIL	NIL	29	NIL	NIL	NIL	21	NIL	NIL	NIL	89	NIL
4th day	NIL	NIL	63	NIL	NIL	NIL	84	NIL	NIL	NIL	231	NIL
6th day	NIL	NIL	148	NIL	NIL	NIL	189	NIL	NIL	NIL	298	NIL
8th day	NIL	NIL	293	NIL	NIL	NIL	298	NIL	38	38	TNTC	87.33
10th day	67	67	TNTC	77.67	NIL	NIL	TNTC	NIL	96	96	TNTC	68

Table 2 reveals that shelf-life study of tomato puree in nanocoated ziplock covers observed that there was no growth of dirrhoeal pathogens from o days till 8th day tested against *E.coli* (MTCC 40) and *S.enterica* (MTCC 3219). On 10th day, it was noticed that there was no microbial growth in *S.enterica* (MTCC 3219).It was found that 78 and 68 percentage of microbial inhibition tested against the *E.coli*(MTCC 40) and *S.dysenteriae* (PSGIMS&R) respectively.

Table 3- Shelf life study of nanocoated infant feeding bottles

Hours	*S.dysenteriae* (PSGIMSR)	
	Experiment (Coated NP)	Control
0 Hr	-	169
½ Hr	-	253
1 Hr	-	TNTC
1 ½ Hr	-	TNTC
2 Hr	23	TNTC
2 ½ Hr	41	TNTC
3 Hr	48	TNTC

From table 3 depicts that the nanocoated infant feeding bottles were tested with milk against *S.dysenteriae* (PSGIMS&R) for every ½ an hour for a period of 3 hours. It was observed that the *S.dysenteriae* were started growing in nanocoated feeding bottles from 2nd hour and on 3hr showed 84 percent of microbial inhibition agsint diarrhoeal pathogens.

Conclusion:

These research findings showed that the (*Glycyrrhiza glabra*) nanoparticles possess natural phenolic, antioxidants, anticancerous and antidiarrhoeal property. Therefore, to avoid the lethal damages of BPA , these silver nanoparticles from plant extracts could be a better alternative as nanocoating in infant feeding bottles and PET bottles. Due to its natural, non-synthetic chemical compounds with low toxicity could be used for various medicinal applications.

Bibliography :

1. Dipanjan Ghosh and Sreeparna Ghosh (2015), Scientific India- Beware of Bisphenol A, Issn 2349-1418, 36-39.
2. Bhan MK. Accelerated progress to reduce under-5 mortality in India. Lancet Glob Health. 2013;1:e172-3.[PubMed]
3. Bassani DG, Kumar R, Awasthi S, Morris SK, Paul VK, et al. Causes of neonatal and child mortality in India: A nationally representative mortality survey. Million Death Study Collaborators. Lancet.2010, 376:1853-60, [PMC free article] [PubMed]
4. Report of the National Commission on Macroeconomics and Health. Estimation of burden of diarrheal diseases in India. In: NCMH Background Papers: Burden of Diseases in India; New Delhi, India: National Commission on Macroeconomics and Health, Ministry of Health and Family Welfare, Govt. of India; 2010, pp. 182-7
5. International Institute for Population Sciences (IIPS) and Macro International. National Family Health Survey (NFHS-3), 2005-06: Mumbai: IIPS; 2007, India. Available from:http://www.rchiips.org/NFHS/report.shtml