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Cytotoxic and Antibacterial Activities of Gossypin

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Abstract – Gossypin, a naturally occurring polyhydroxy flavonoid, when subjected to *in vitro* cytotoxic screening against vero cell lines exhibited 68.75% inhibition at a concentration of 1000 µg. When tested against five bacteria and five fungi, the flavone derivative showed a moderate activity against *Staphylococcus aureus* and *Escherichia coli*, mild inhibition against *Pseudomonas aerugenosa* and *Salmonella typhi* and no activity against any of the tested fungi, in the concentrations studied.
Keywords – Gossypin, cytotoxicity, antibacterial activity

Introduction

Flavonoids are widely distributed in nature and exhibit a broad spectrum of biological activities (Middleton and Kandaswami, 1993; Evans *et al.,* 1997). They also exert inhibitory actions against various enzymes (Formica and Regelson, 1995). Gossypin is a naturally occurring polyhydroxy flavone-glycoside present in the flowers of *Hibiscus vitifolius* Linn. (Family: Malvaceae). Earlier studies on gossypin revealed significant anti-inflammatory (Parmar and Ghosh, 1980), analgesic (Viswanathan *et al.,* 1984 & 1985), hepato-protective (Anon *et al.,* 1992) and hypoglycemic (Raghunathan and Sulochana, 1994) activities. It was also reported to reduce the toxicity of dermally induced lipid peroxidation (Vijayaraghavan *et al.,* 1991) and delay the onset of cataract formation in rats (Parmar and Ghosh, 1979). As many flavonoids were not screened for cytotoxic and antibacterial activities, gossypin which is obtained in good yield, has been subjected to these studies in our work. In this communication, the cytotoxic and antibacterial activities of gossypin are reported.

Experimental

Isolation of gossypin – Gossypin was isolated by the procedure of Viswanathan *et al*. (1984). About 500 g of shade dried and coarsely powdered flowers of *H. vitifolius* were exhaustively extracted with 90% ethanol for 48 hours. The solvent was distilled-off over hot water bath and traces of ethanol were removed by concentration *in vacuo*. The extract on purification over a column of silica gel yielded gossypin (m.p. 228°; Yield: 0.09% w/w).

Determination of cytotoxicity – Vero cell lines were obtained from Tamil Nadu Veterinary and Animal Sciences, Chennai, India. All the reagents and media were maintained at 37 °C before use.

The cell monolayer, prepared as per the method of Freshney (1987), was trypsinised and the cells were counted. 0.1 mL of these cells were seeded in the wells of microtitre plates (1×10^4 cells / well) in triplicate and incubated. After 24 h, 0.1 mL of gossypin in concentrations of 10, 100 and 1000 µg were added to the cells in microtitre plates and incubated for 72 h. The control wells were maintained only with growth medium. After 72 h, the cells were assessed for signs of toxicity such as swelling, shrinkage, granularity, floating, vacuolization and other morphological changes. After the medium was discarded, the cells were trypsinized with 50 µL TPVG for 3 - 4 min and 50 µL of minimum essential medium was added to stop the trypsin activity. Then the cells were pooled out and the percentage inhibition was calculated using the formula,

$$\text{Percentage inhibition} = 100 - \frac{\text{Total number of cells after drug treatment}}{\text{Total number of cells in control}} \times 100$$

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Determination of Cell metabolic function by protein synthesis – The cells from the wells were trypsinised using 100 µL trypsin, centrifuged at 5000 rpm for 10 minutes. The protein level was estimated by the method of Lowry *et al*. (1951). The percentage inhibition of the growth was calculated using the equation,

$$\text{Percentage inhibition} = 100 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Cytopathological studies – The cytopathological changes were observed at all concentrations and photomicrographs were taken with NIKON-LAB PHOT-2 camera.

Antimicrobial studies – The antibacterial and antifungal activities were carried out by Cup-plate method (Cruickshank, 1968) using five bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aerugenosa*, *Salmonella typhi* and *Klebsiella pneumoniae)* and five fungal strains *(Candida albicans*, *Rhizopus sp., Aspergillus niger, Aspergillus flavus* and *Penicillium sp.).* Gossypin was dissolved in DMSO and used in the concentrations of 0.5, 1.0, and 2.0 mg. All bacterial cultures were grown in nutrient agar medium and the antibacterial activity was studied after 24 hours of incubation. The fungal cultures were grown in Sabouraud's dextrose agar medium and the antifungal activity was studied after 48 h of incubation. Ciprofloxacin (10 µg) and Clotrimazole (100 µg) were used as standards for antibacterial and antifungal tests respectively. Minimum inhibitory concentration (MIC) study was carried out by double dilution method (Kavanagah, 1972) for those microbes which gave significant zone of inhibition.

Results

Gossypin showed a concentration-dependent inhibition of cell growth (Table 1). The percentage inhibition was found to be 41.66, 60.42 and 68.75 respectively in the concentrations of 10, 100 and 1000 µg of gossypin.

The protein content was found to decrease with increase in the concentration of gossypin (Table 2). The percentage inhibition of protein synthesis was found to be 4.94, 20.99 and 67.9 in the gossypin concentration of 10, 100 and 1000 µg respectively.

Gossypin treatment showed a well-defined pathological change on vero cell line. At 10 µg/mL concentration the cells were damaged, rounded and shrunken. Grouping and giant cell formations were also observed. Nearly 70% of the cells were found to be viable. At 100 µg concentration, 50% confluency and giant cell formation were observed.

Table 1. Determination of cytotoxicity by tryphan blue dye exclusion method in vero cell line

Concentration µg/mL	Average number of cells in the wells			% inhibition
	Viable count	Dead count	Total count	
Control	24.0	0	24.0	0
Gossypin				
10	9.0	5.0	14.0	41.66
100	3.0	6.5	9.5	60.42
1000	0.5	7.0	7.5	68.75

Table 2. Determination of cell metabolic function by protein synthesis in vero cell line

Drug concentration (µg/mL)	Absorbance (λ_{max})	% inhibition	CTC 50%
Control	0.081	0	
Gossypin			
10	0.077	4.94	
100	0.064	20.99	600 µg
1000	0.026	67.90	

At 1000 µg, the cells became rounded, shrunken, uneven and particulated and vacuolated with grouping and peeling of monolayer cells. The control cells showed 100% confluency (Fig. 1).

In the antimicrobial studies, gossypin exhibited moderate activities against *S.aureus* and *E.*coli and mild inhibitions against *P.aerugenosa and S.typhi* when compared to the standard drug Ciprofloxacin. Its effect on the other bacteria and the tested fungi were not significant in the concentrations employed (Table 3).

Discussion

Vero cell line, a fibroblast type, initiated from the kidney of normal adult African green monkey is employed extensively in virus replication studies and plaque assays. The cells are used for the assay in the present study were SV-40, SV-5, measles, arbovirus, rubella, Simian-adeno and polio viruses. Gossypin showed a pronounced cytotoxic action by inhibiting cell proliferation and finally killing the cells which was well implicated by the uptake of tryphan blue dye by the dead cells. The cell death was up to 68% suggesting that gossypin could be a good cytotoxic agent.

The cell metabolic function was assessed by estimating the protein level. The intensity of blue colour depends upon the amount of aromatic amino acids present. The greater the cell metabolic function greater is the intensity. Gossypin exhibited a reduced cell activity when compared

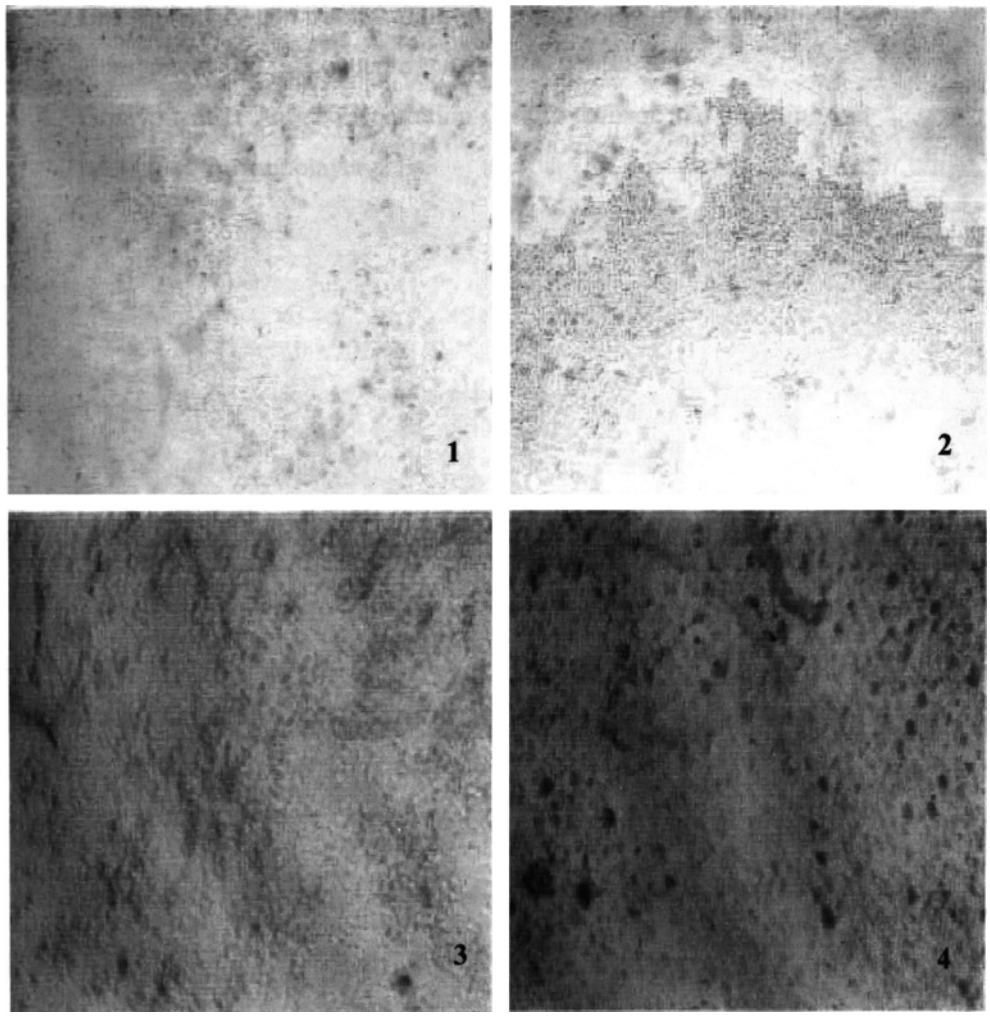

Fig. 1. Cytopathological changes of gossypin on vero cell lines.
1. Control showing 100% confluency, 2. Gossypin at 10 µg concentration showing grouping and giant cell formation, 3. Gossypin at 100 µg concentration showing 50% confluency, 4. Gossypin at 1000 µg concentration showing 90% damage and grouping and peeling of the monolayer cells.

Table 3. Antimicrobial activity of gossypin

Microbes	Average zone of inhibition (mm)				
	0.5	1.0	2.0	Standard	MIC
Bacteria					
Staphylococcus aureus	14.25	16.7	27.25	27.6	0.25
Escherichia coli	12	12.5	14.5	36.3	0.5
Pseudomonas aerugenosa	10.5	11.5	12.75	31.3	0.5
Salmonella typhi	–	–	10.0	25.0	1.5
Klebsiella pneumoniae	–	–	–	32.7	–

Standard-Ciprofloxacin 10 µg
Diameter of well-7 mm
MIC values in mg

to the control. At 1000 µg concentration, reduction in the cell metabolism was maximum suggesting the efficacy of gossypin. The 50% inhibition of cell metabolism was found to be 600 µg of gossypin.

The well defined pathological changes such as giant cell formation, rounded and shrunken appearance of cells, particulated and vacuolated structure, grouping and peeling of monolayer observed on gossypin treatment further confirmed the compound's potential as a cytotoxic agent against the vero cell line.

In the antimicrobial studies, gossypin showed a moderate activity against *S. aureus* and *E. coli*, mild inhibition against *P. aerugenosa* and *S. typhi* and virtually no activity against *K.pneumoniae* and the tested fungal strains in the concentrations employed suggesting that in low concentrations the compound is not effective and

probably in higher concentrations the compound might give significant results.

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