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Genotoxicity Studies of Magiferin Isolated from *Salacia chinensis* Linn

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Abstract: Roots of *Salacia chinensis* have been used as an antidiabetic drug in the indigenous system of medicine. The root bark contains diketones, fatty matter, rubber, dulcitol, mangiferin, phlobatannin and glycosidal tannins. Roots are astringent, abortifacient and a decoction is useful in amenorrhoea, dysmenorrhoea and venereal disorders. The plant was evaluated with a series of genotoxicity studies in order to confirm the safety of its usage. It showed no mutagenicity up to 5 mg/plate when tested with *Salmonella typhimurium* TA97a, TA98, TA100, TA102 and TA1535 strains with or without metabolic activation. On the other hand Magiferin from *Salacia chinensis* Linn shows a significant protective effect against mutagenicity induced by mutagen in *S. typhimurium* TA98 and TA100 strain with or without metabolic activation. Similarly, *in vitro* chromosomal aberration assay did not reveal any significant alterations up to 5 mg/culture as compared to the negative control both in the presence and absence of the metabolic activation (S9 mix). The results of these studies indicate that Magiferin from *Salacia chinensis* Linn is non-mutagenic in AMES test, exhibit protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain and non-clastogenic in *in-vitro* chromosomal aberration study.

Key words: Magiferin from *Salacia chinensis* Linn · Chromosomal aberration study · Mutagenicity · *Salmonella typhimurium*

INTRODUCTION

Salacia chinensis is an important medicinal plant belonging to the family Hippocrateaceae. It is a small erect or straggling tree or large, woody, climbing shrub found almost throughout India including Andaman and Nicobar Islands. The plant and its extracts have been evaluated for number of activities like anti-inflammatory, cardio-tonic, sedative and neuron-muscular. Mangiferin is a xanthone glucoside and an active phytochemical present in common principal constituent of *Salacia* species [1]. *Salacia chinensis* Linn., a member of the family Hippocrateaceae. The roots are used in indigenous system of medicine and treated for diabetic, astringent, abortifacient, amenorrhoea, dysmenorrhoea and veneral diseases. Mangiferin is called as C-glucosyl xanthone [2] and 2-â-D-glucopyranosyl-1, 3,6,7-tetrahydroxy xanthone [3]. Mangiferin is recommended to treat immuno-deficiency diseases such as diabetes, hepatitis, arthritis, cardiac and mental disorders [4]. The aqueous extract of *Mangifera indica* leaves possesses antihyperglycemic activity in glucose-induced hyperglycemic rats and mice [5-6]. The mangiferin exerts antidiabetic properties by decreasing insulin resistance in non-insulin dependent KK/Ay mice [7]. The chronic administration of mangiferin significantly improved oral glucose tolerance in glucose-loaded normal rats [8].

Salacia chinensis is an important medicinal plant belonging to the family Hippocrateaceae. *S.chinensis* is a small erect or straggling tree or large, woody, climbing

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shrub found almost throughout India including Andaman and Nicobar Islands, thriving along seashore and river banks as well as in forests. The fruit is one to two centimeters in diameter and red when ripe. Ripe fruits are eaten. Roots have been used as an antidiabetic drug in the indigenous system of medicine and clinical tests substantiated their efficacy. The root bark contains diketones, fatty matter, rubber, dulcitol, mangiferin, phlobatannin and glycosidal tannins. Roots are astringent, they are said to be abortifacient and a decoction is useful in amenorrhoea, dysmenorrhoea and venereal disease. It is also known as Dimal, modhuphal in Bengali, Ingli, nisul-bondi in Marwari. The important species of *Salacia* are *S. chinensis*, *S. reticulata*, *S.malabaricia*, *S. macrosperma*, *S. beddomi*, *S. oblonga* and *S. fruticosa*. The roots of *S. chinensis* have been used as an antidiabetic drug, astringent, abortifacient and root decoction used to treat amenorrhoea, dysmenorrhoea and venereal diseases. The stems of *S.chinensis* have been extensively used for carminative, emmenagogue, blood tonic, cardio tonic, anti-inflammatory, antidiabetic purposes and the treatment of rheumatism, leukorrhea and stimulated lochial excretion. The stem and leaves are commercially used as a gutt-a-linear isomer of natural rubber. The roots and stem of *S. reticulata* have been used for the treatments of rheumatism, gonorrhea, skin diseases and initial stages of diabetes in the Ayuredic system of Indian traditional medicine. The decoction of *S.reticulata* root is used in the treatment of rheumatism, gonorrhea, itching and swelling, asthma, thirst, amenorrhea and dysmenorrhea. The S. *reticulata* collected in Sri Lanka and *S. oblonga* collected in India were found to show hypoglycemic effects in oral sucrose and maltose-loaded rats and á-glucosidase inhibitory activities against sucrase, maltase and isomaltase [9-11].

MATERIALS AND METHODS

Plant Materials and Chemicals: The roots of *Salacia chinensis* were collected from Veenangaputtu, Karumpakkam, Thangal and Kurumpuram (all areas are nearest to Pondicherry). The plant voucher specimen (778) has been deposited in Centre for Advanced Studies in Botany, University of Madras.

Preparation of Plant Extract: The roots of *Salacia chinensis* were washed thoroughly with tap water, shade dried, cut into small pieces and were crushed to moderately coarse powder. It was extracted using 95% methanol in soxhlet apparatus for 6h.The extract was concentrated by using rotary evaporator at 40-50º C under reduced pressure. The methanolic root extract yield was 23.5%. Commercially available per-coated thin layer chromatography aluminium sheets with slicagel $60F_{254}$ were used. About 50µl of authentic mangiferin and menthanolic root extract of *S. chinensis* were applied as a single spot at the base of the plate. The plates were developed at room temperature in a chromatogram tank containing the solvent system ethyl acetate: formic acid: galacial acetic acid: water in the ratio of 100:11:11:2.4. The chromatogram was air-dried and viewed in an UV light at 365 nm.

Purification of Mangiferin by Column Chromatography: A portion of the crude methanol extract was subjected to column chromatography over slicagel with chloroform gradient elution using ethyl acetate in methanol 95:5:85:10 totals of 14 elute (50ml) were collected and combined in to basis of their TLC similarities. The mangiferin yield was eluted on the fraction ratio of 60:40 (ethyl acetate: methanol), which was confirmed by TLC analysis. The concentrated fraction was subjected recolumn chromatography, while purity was confirmed by using HPLC analysis. The yield of mangiferin was 7.8%.

Purity Analysis of Mangiferin: High Performance Liquid Chromatography was performed to confirm the purity of the mangiferin [12] and C18 column was used to separate the mangiferin. The mobile phase of an isocratic consisting of acetonitrile and 3% of acetic acid (16:84) was used with a flow rate of 0.5 ml/min and the UV-Visible detector wavelength was set at 254 nm. The authentic mangiferin was purchased from Sigma Aldrich Company showed a single peak (100 %). The isolated mangiferin was closely resampled with authentic purity that was greater than 99.4 % (w/w).

Chemicals: Dimethyl sulfoxide (DMSO-CAS No. 67-68-5), nicotinamide adeninedinucleotide phosphate sodium salt (NADP-CAS No. 214-664-6), D-glucose-6-phosphate disodium salt (CAS No. 3671-99-6), L-histidine monohydrate (CAS No. 7048-02-4), D-Biotin (CAS No 58-85-5) were purchased from Sigma Chemical Co. and minimal essential medium (MEM CAT No. 41090-036) was procured from Gibco. The S9 microsome fraction was prepared in house from the livers of rats treated with sodium phenobarbitol.

Standard Mutagens: 2-aminofluorene (CAS No 613-13-8), Mitomycin C (CAS No 56-07-7), 4-nitroquinolene-1-oxide (CAS No 56-57-5), sodium azide (CAS No 26628-22-8), Benzo(a)pyrene (CAS No 200-028-5) were also obtained from Sigma. Oxoid nutrient broth No. 2 (Oxoid) and Difco bacto agar (Difco) were used for the preparation of bacterial growth media.

AMES Assay: S. typhimurium strains TA97a, TA98, TA100, TA1535 and TA102 were obtained from Bruce Ames Laboratory, Molecular and Cell Biology, University of California and checked for their viable counts and genotype characteristics. Plate incorporation method Maron and Ames, 1984 using histidine-dependent strains of *S. typhimurium* TA97a, TA98, TA100, TA102 and TA1535 in the presence and absence of metabolic activation system (S9 liver fraction) was adopted for assessing the mutagenicity. Mangiferin of *S.chinensis* was tested for its mutagenic properties at five different concentrations viz., 5, 2.5, 1.25, 0.625 and 0.312 mg/plate. 100 µl of various concentrations of Mangiferin of *S.chinensis* dissolved in DMSO were added to 2 ml top agar mixed with 100µl of bacterial culture and then poured on to a plate containing minimal glucose agar. These plates were incubated at 37^0C for 48 h and his+ revertant colonies were manually counted and the results were shown as the mean of the two plates with standard deviation. The influences of metabolic activation were tested by adding 500 µl of S9 mixture. The experiments were analysed in triplicate and was repeated to confirm the result. The criteria employed to interpret the results of Ames test as positive were similar to those used in regulatory guidelines OECD test guideline No. 471(1997) [13-14]. The number of induced mutation should be at least twice the activity observed in negative control and there must be a reproducible dose response curve. Concurrent positive and negative (DMSO) controls were used in the study. The standard mutagens used as positive controls in each experiment were without metabolic activation, 4-nitroquinoline-1-oxide (5µg/plate) for strain TA97a and TA98, sodium azide (5µg/plate) for strain TA100 and TA1535, mitomycin-C (0.02mg/plate) for TA102. In case of positive controls with metabolic activation, 2-aminoflurene (20µg/plate) for TA97a, TA98, TA100, TA1535 and TA102 were used.

Anti-Mutagenicity Test: Based on the results of mutagenicity testing, Mangiferin of *S.chinensis* were tested for its anti-mutagenic properties [15] at five different concentrations viz., 5, 2.5, 1.25, 0.625 and 0.312 mg/plate. Dimethyl sulphoxide (DMSO) was used as solvent control. The S9 mix (500 µl) or phosphate buffer for the presence and absence of metabolic activation, 100 µl of the respective positive control (without metabolic activation sodium azide for TA100 and 4-nitroquinolene-1-oxide for TA98 in case of with metabolic activation 2-aminofluorene for both the strains), 100 µl of the appropriate concentration of the extract, 100 µl of respective bacterial culture, were added to sterile capped tubes and incubated in an incubator for 30 m at 37 ± 1°C. After incubation, the mixture was added to sterile tubes containing 2 ml of top agar kept at 45 ± 2 °C in a water bath. The tubes containing the mixture and top agar were gently mixed and then overlaid onto the surface of minimal glucose agar plates prepared under aseptic conditions contained in 100 × 10 mm plate. After solidification, the plates were inverted and incubated at 37 ± 1°C for 48-72 h. Plating was done in duplicates. Positive and negative control (DMSO) plates were also prepared in duplicates. The inhibition rate of mutagenicity (%) was calculated with respect to the number of revertant colonies in the control group treated with the corresponding mutagen by the following assay [16].

RESULTS

All the strains of *S. typhimurium* viz., TA97a, TA98, TA100, TA102 and TA1535, exposed to different concentrations of *B. monnieri*, did not show two-fold or greater increase in the mean number of revertants as compared to the negative control group as given in Table 1. All strains used in the study exhibited marked increase (>10-fold) in the number of revertants when treated with positive control agents. The results confirmed the sensitivity of the tester strains to mutagens and thus the validity of the assay. The results indicated that the mean number of histidine revertants in the treatment groups were comparable to the mean number of revertants in the negative control group in all the five *S. typhimurium* tester strains viz., TA97a, TA98, TA100, TA102 and TA1535 both in the absence and presence of metabolic activation. Mangiferin of *S.chinensis* upto 5mg/plate in the presence and absence of metabolic activation was found to be non-mutagenic to all the five *S. typhimurium* tester strains. On the other hand, ethanol extract of Mangiferin of *S.chinensis* showed a significant dose dependent anti-mutagenic activity,

Table 1: Mutagenic activity of Ethanol extract of Mangiferin from *Salcia chineases*

	Revertant Colonies / plate (mean 9n=3) ± S. D.)									
	TA97a		TA98		TA1535		TA100		TA102	
Dose Level (mg/plate)	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
NC (DMSO)	184 ± 5	187 ± 4	20 ± 2	20 ±3	13 ± 2	14 ± 2	181 ± 2	184 ± 6	294 ± 6	304 ± 6
5	178 ± 3	184 ± 6	20 ± 2	20 ± 1	12 ± 2	10 ± 1	186 ± 4	183 ± 7	299 ± 9	298 ± 2
2.5	178 ±2	187 ± 3	19 ± 2	20 ± 2	13 ± 3	12 ± 1	186 ± 5	182 ± 5	298 ± 2	302 ± 3
1.25	178 ± 7	188 ± 2	20 ± 2	21 ± 2	14 ± 2	12 ± 1	184 ± 6	180 ± 3	298 ± 3	304 ± 5
0.625	179 ± 3	188 ± 3	19 ± 2	21 ± 2	11 ± 1	13 ± 2	184 ± 6	183 ± 3	296 ± 5	298 ± 2
0.312	178 ±5	186 ± 5	20 ± 1	20 ± 1	14 ± 2	12 ± 2	186 ± 4	185 ± 5	299 ± 8	302 ± 4
PC SA	NA	NA	NA	NA	1152 ± 27	NA	2059 ± 15	NA	NA	NA
PC 4NQNO	1345 ± 25	NA	1428 ± 23	NA	NA	NA	NA	NA	NA	NA
PC MMC	NA	NA	NA	NA	NA	NA	NA	NA	3046 ± 39	NA
PC 2AF	NA	2152± 25	NA	1338 ± 16	NA	681 ± 8	NA	2165 ± 17	NA	3129±27

Key: µg = microgram, S.D. = Standard deviation, NC = Negative control, DMSO= Dimethylsulfoxide, PC = Positive control, 4NQNO = 4-Nitroquinolene N Oxide, SA = Sodium azide, MMC = Mitomycin C, 2AF = 2Aminofluorene, NA = Not Applicable, n = No. of replicates

Table 2: Inhibition of Mutagenicity by Magiferin in *S. typhimurium* TA98 assay system

	His+ Revertant colonies / plate (mean ± S.D.)			
Dose (mg/plate)	Presence of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	21 ± 2	-	22± 2	-
0.312	1020 ± 4	33	564 ± 4	32
0.625	819 ± 4	46	487 ± 4	42
1.25	665 ± 5	56	408 ± 2	52
2.5	87 ± 3	95	61 ± 2	95
5	21± 3	100	22 ± 4	100
PC	1501 ± 4	-	824 ± 4	-

Key: NC= negative control, PC= positive control

Table3: Inhibition of Mutagenicity by Magiferain in *S. typhimurium* TA100 assay system

	His+ Revertant colonies/plate (mean ± S.D.)			
Dose (mg/plate)	Presence of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	179 ± 20	-	158 ± 13	-
0.312	635 ± 17	62.66	492 ± 41	75.12
0.625	555 ± 30	69.21	380 ± 25	83.46
1.25	239 ± 25	95	300 ± 31	89.42
2.5	221± 16	96.57	250 ± 28	94.47
5	186 ± 11	100	148 ± 25	100
PC	1400 ± 28	-	1500 ± 69	-

Key: *NC= negative control, PC= positive control*

in *S. typhimurium* TA98 and TA100 strain with or without metabolic activation which is shown in Table 2 and 3. Mangiferin of *S.chinensis* exhibit protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain.

DISCUSSION

The results of anti-mutagenic activities showed that the extract was highly effective in reducing the mutagenicity caused by the mutagen 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene (Table 2 and 3). The mutagen 2 Aminoflurone works by causing framshift

mutation by forming adducts on the C8 position of guanine in DNA in presence of microsomal activation. In contrast sodium Azide requires no activation by hepatic microsomal enzymes to damage DNA and induce mutagenicity. This suggests that this extract may inhibit microsomal enzymes activation or that they may directly protect DNA strands from the electrophilic metabolites of the mutagen. Many triterpenoids have antioxidants depending on the redox potential, either accept or donate electrons, which may alternatively reduce them protective against mutagen. Our results from these experiments on antimutagenicity suggest that triterpenoids might contain antioxidants which protects from the mutagens.

Our purpose was to investigate the possible mutagenic, anti-mutagenic properties of *mangiferin* extracts with Ames assay and Chromosomal aberration. The result obtained is ethanol extract of *Mangiferin from Salcia chineases* is non-mutagenic upto 5mg/plate both in the presence and absence of S9 (Table 1). Results of anti-mutagenic activities showed that the ethanol extract of *Mangiferin from Salcia chineases* were highly effective in reducing the mutagenicity caused by the mutagen 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene (Table 2 and 3). These features make ethanol extract of *mangiferin* a promising candidates for further studies. However, *in-vivo* studies are in progress.

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