International Multidisciplinary Research Journal 2011, 1/1:01-05
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IRMJ- Botany

Antimutagenicity of mangiferin purified from *Salacia chinensis* Linn.

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Abstract: Mangiferin is a xanthone glucoside and an active phytochemical present in common principal constituent of *Salacia* species. Mangiferin was purified from methanolic root extract of *Salacia chinensis*. Mangiferin is recommended to treat immuno-deficiency diseases such as diabetes, hepatitis, arthritis, cardiac and mental disorders. Mangiferin was evaluated for antimutagenicity studies in order to confirm the safety of its usage.

Mangiferin 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 mangiferin showed a significant protective effect against mutagenicity induced by mutagen in *S. typhimurium* TA98 and TA100 strain with or without metabolic activation. The results of these studies indicate that mangiferin 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.

Keywords: *Salacia chinensis*; Antimutagenicity; Mutagenicity; *Salmonella typhimurium*; Mangiferin; Ames test.

INTRODUCTION

Mangiferin is an active phytochemical present in common principal constituent of *Salacia* species (Yoshikawa et al., 2001). The *Salacia chinensis Linn* is an important medicinal plant belonging to the family Hippocrateaceae. This is a small erect or straggling tree or large, woody, climbing shrub found almost throughout India including Andaman and Nicobar Islands, thriving along seashore and river banks as well as in forests. 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.

Mangiferin is called as C-glucosyl xanthone (Scartezzini and Speroni 2000) and 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy xanthone (Muruganandan et al., 2002). Mangiferin is recommended to treat immuno-deficiency diseases such as diabetes, hepatitis, arthritis, cardiac and mental disorders (Sanchenz et al., 2000). Mangiferin exerts antidiabetic properties by decreasing insulin resistance in non-insulin dependent KK/Ay mice (Miura et al., 2001). The chronic administration of mangiferin significantly improved oral glucose tolerance in glucose-loaded normal rats (Muruganandan et al., 2005). The mangiferin possess antidiabetic activity against STZ – induced diabetic rats (Periyar selvam et al., 2009).

The pharmacology of the mangiferin is gaining increased attention in the recent years owing to its modulatory functions on oxidative mechanisms in various disorders (Sanchez et al., 2000; Muruganandan et al., 2002; Garrido et al., 2004; Andreu et al., 2005; Jagetia and Venkatesha, 2005). The mangiferin is as well shown to exhibit antitumor (Guha et al., 1996), antiviral (Zheng and Lu, 1990; Zhu et al., 1993; Yoosook et al., 2000), immunomodulatory (Guha et al., 1996; Makare et al., 2001; Leiro et al., 2004) and radioprotective (Jagetia and Baliga, 2005) activities under different experimental conditions.

The antimutagenic agents or substances were found in vegetables, fruits, and medicinal plants. The antimutagenic compounds, such as fiber, cinnamaldehyde, coumarin, vaniline, epigalocatechin gallate, and tannic acid were isolated from plants (Kada, 1982). Since there is no evidence of antimutagenicity data available for mangiferin, hence we have investigated the mutagenic activity and anti-mutagenicity of mangiferin, an important active compound present in different medicinal plant species. They were confirmed by Ames test and chromosomal aberration test based on Organisation for Economic Co-operation and Development (OECD) guidelines.

Received: April 28, 2011; Revised May 17, 2011; Accepted May 17, 2011.

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MATERIAL AND METHODS

Plant materials collection

The roots of *Salacia chinensis* were collected from Veenangaputtu, Karumpakkam, Thangal and Kurumpuram (all areas are nearest to Puducherry). 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 *S. 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 45 - 50 ºC under reduced pressure (Periyar selvam et al., 2009).

Purification of mangiferin by Column chromatography

A portion of the crude methanolic root extract was subjected to column chromatography over slicagel with chloroform gradient elution using ethyl acetate in methanol 95:5 - 15:85 respectively for the total amount of 17 elutes (100 ml) were collected and combined in to basis of their Thin Layer Chromatography (TLC) similarities. The mangiferin yield was eluted on the fraction ratio of 40:60 (ethyl acetate: methanol), which was confirmed by TLC analysis. The concentrated fraction was subjected to recolumn chromatography, while purity was confirmed by using high performance liquid chromatography analysis (Periyar selvam et al., 2009)

Purity analysis of mangiferin

High Performance Liquid Chromatography was performed to confirm the purity of the mangiferin (Geodakyan et al., 1992) 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, when compared with authentic sample; the isolated mangiferin was closely resembled with authentic purity that was found to be 99.4% (w/w).

Chemicals

Dimethyl sulfoxide (DMSO-CAS No. 67-68-5), nicotinamide adenine dinucleotide 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. The S9 microsome fraction was prepared in house from the livers of rats treated with sodium phenobarbitol (Venitt et al., 1990).

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), oxoid nutrient broth No. 2 (Oxoid) and difco bacto agar (Difco) were used for the preparation of bacterial growth media.

Ames assay

The strains of *S. typhimurium* 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. The 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 was tested for its mutagenic properties at five different concentrations viz., 5, 2.5, 1.25, 0.625 and 0.312 mg/plate. The various concentrations (100 µl) of mangiferin was dissolved in DMSO and added to 2 ml top agar and then mixed with 100 µl of bacterial culture, finally poured on to a plate containing minimal glucose agar. These plates were incubated at 37 ^{o}C 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, that was OECD test guideline No. 471(1997). 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 (5 µg/plate) for strain TA97a and TA98, sodium azide (5 µg/plate) for strain TA100 and TA1535, mitomycin-C (0.02 mg/plate) for TA102. In case of positive controls with metabolic activation, 2-aminoflurene (20 µg/plate) for TA97a, TA98, TA100, TA1535 and TA102 were used.

Anti-mutagenicity test

Based on the results of mutagenicity testing, mangiferin were tested for its anti-mutagenic properties (Lee et al., 2000) 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 compound, 100 µl of respective bacterial culture were added to sterile capped tubes and incubated for 30 min at 37 ± 1 °C. After incubation, the mixture was added to sterile tubes containing 2 ml of top agar and 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 ºC 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 (Hyder et al., 2007).

RESULTS

All the strains of *S. typhimurium* viz., TA97a, TA98, TA100, TA102 and TA1535, exposed to different concentrations of mangiferin, 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 up to 5 mg/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, m*angiferin* showed a significant dose dependent anti-mutagenic activity, in *S. typhimurium* TA98 and TA100 strain with or without metabolic activation, which is shown in Table 2 and 3. Mangiferin exhibit protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain.

Table - 1 Mutagenic activity of mangiferin

Dose concentration (mg/plate)	Revertant Colonies / Plate [Mean (n=3) ± S.D]									
	TA97a		TA98		TA1535		TA100		TA102	
	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)
NC (DMSO)	176 ± 4	179 ± 4	18 ± 2	18 ±1	18 ± 3	19 ± 1	176 ± 8	176 ± 4	301 ± 4	299 ± 4
0.313	176 ± 5	178 ± 2	16 ± 2	15 ± 1	18 ± 3	18 ± 2	174 ± 6	171 ± 3	303 ± 9	298 ± 8
0.625	175 ± 4	180 ± 1	17 ± 2	18 ± 3	19 ± 2	18 ± 2	172 ± 6	169 ± 4	296 ± 3	298 ± 3
1.25	181 ± 2	178 ± 2	14 ± 2	17 ± 3	18 ± 1	18 ± 1	174 ± 6	170 ± 2	302 ± 3	299 ± 2
2.5	183 ± 2	180 ± 4	19 ± 1	18 ± 2	18 ± 2	17 ± 1	175 ± 6	171 ± 2	301 ± 4	304 ± 3
5	182 ± 3	182 ± 2	18 ± 2	20 ± 1	17 ± 2	19 ± 1	171 ± 2	171 ± 1	302 ± 6	301 ± 3
PC SA	NA	NA	NA	NA	115 ± 27	NA	201 ± 15	NA	NA	NA
PC 4NQNO	134 ± 25	NA	142 ± 23	NA	NA	NA	NA	NA	NA	NA
PC MMC	NA	NA	NA	NA	NA	NA	NA	NA	304 ± 39	NA
PC 2AF	NA	2152 ± 25	NA	1338 ± 16	NA	681 ± 8	NA	2165 ± 17	NA	3129 ± 27

Key: mg = milligram, S.D = Standard Deviation, NC = Negative Control,
DMSO = Dimethyl Sulfoxide, 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 mangiferin in *S. typhimurium* TA98 assay system

Dose Concentration (mg/plate)	His+ Revertant Colonies / Plate (Mean ± S.D.)			
	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

Table - 3 Inhibition of mutagenicity by mangiferin in *S. typhimurium* TA100 assay system

Dose Concentration (mg/plate)	His + Revertant Colonies / Plate (Mean ± S.D.)			
	Presence of S9 Mix	Inhibition of mutagenesis (%)	Absence of S9 Mix	Inhibition of mutagenesis (%)
NC (DMSO)	178 ± 2	–	170 ± 3	–
0.312	638 ± 3	63	495 ± 3	75
0.625	552 ± 7	70	382 ± 4	84
1.25	240 ± 3	95	301 ± 7	88
2.5	225± 4	96	257 ± 5	93
5	183 ± 4	100	170 ± 2	100
PC	1423 ± 5	–	1500 ± 69	-

Key NC = Negative Control, PC = Positive Control

DISCUSSION

The antimutagenic activity of mangiferin isolated from *S. chinensis* was performed by Ames test with *S. typhimurium* strains. In this test we found out that mangiferin have a strong antimutagenic activity; the number of revertants induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene and mangiferin decreased almost completely. The antimutagenic activity of mangiferin on the activity of standard mutagens was more potent. The mangiferin probably act as desmutagens, which directly inactivate positive mutagens or inhibit their metabolic activation.

In conclusion, we found out that mangiferin has potential inhibitory effect on mutagen, based on the Ames test. The Ames test results revealed mangiferin had no mutagenic potential towards tester strains, instead of that it has potent antimutagenic activity. The mangiferin might be included in the group of natural antimutagens.

ACKNOWLEDGEMENT

The authors are grateful to Prof. R. Rengasamy, Director, CAS in Botany, University of Madras.

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