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Synthesis and evaluation of novel analogues of mangiferin as potent antipyretic

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ABSTRACT

Objective: To screen different analogues of mangiferin pharmacologically for antipyretic activity. **Methods:** The naturally occurring xanthone glycoside mangiferin was isolated by column chromatography from the ethanolic extract of stem bark of *Mangifera indica*. Mangiferin was further converted to 5-(N-phenylamino methyleno) mangiferin, 5-(N-p-chlorophenylamino methyleno) mangiferin, 5-(N-2-methyl phenylamino methyleno) mangiferin, 5-(N-p-methoxy phenylamino methyleno) mangiferin, 5-(N, N-diphenylamino methyleno) mangiferin, 5-(N-α-napthylamino methyleno) mangiferin and 5-(N-4-methyl phenylamino methyleno) mangiferin analogues. The synthesized compounds were further screened for antipyretic activity along with mangiferin at a dose level of 100 and 200 mg/kg. Mangiferin and its analogues were characterized by melting point and R_f value determination and through spectral technique like UV, IR, and NMR spectral analysis. **Results:** The antipyretic activity of mangiferin as well as all analogues was found to be more significant in at higher dose *ie.* 200 mg/kg which was depicted through a decrease in rectal temperature up to 3 h. **Conclusions:** The antipyretic activity of mangiferin and its analogues may be attributed to inhibition in synthesis of TNF-α and anti-oxidant activity associated with amelioration of inflammatory actions of cytokines.

1. Introduction

Mangiferin, $C_{19}H_{18}O_{11}$, a glucoxanthone (1,3,6,7-tetrahydroxyxanthone-C_2-β-D-glucoside) has been reported to be present in various parts of *Mangifera indica* viz leaves[1], fruits, stem bark, roots[2] and heartwood[3]. Mangiferin has attracted considerable interest in view of its numerous pharmacological activities, including antibacterial[3], antitumor, immunomodulatory and antiHIV[4], antidiabetic[5], antioxidative[6], anthelminthic, antiallergic[7] and antiinflammatory activity[8], antiviral[9], inducer of macrophage activation[10]. In Cuba, mangiferin is traditionally used as an antiinflammatory, analgesic and also as an antioxidant under brand name Vimang®. In Sri Lanka, mangiferin is used in the obesity treatment and particularly for diabetes type Ⅱ under brand name Salaretin®.

Updated literature survey reveals that much attempts have not been made to make the derivatives of mangiferin. Secondly, amino methylation of xanthone glycoside is one method which has yielded derivatives of xanthone glycosides successfully. Working on the same guidelines, it was decided to subject mangiferin also to this chemical modification *ie.* aminomethylation. Further, unlike anti-inflammatory and analgesic property, the possible antipyretic activity of neither mangiferin nor its derivatives has been studied to our best knowledge. This prompted us to investigate upon mangiferin and its derivatives for their antipyretic activity which can possibly open a new therapeutic use of these compounds.

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2. Materials and methods

The stem bark of *Mangifera indica* cultivar desi which was collected from saunda village Modinagar, Ghaziabad district of U.P. in the month of April 2006, was authenticated at the Department of Botany, M. M. P. G. College, Modinagar. Melting points were determined in open capillary tubes and purity of the compounds was checked by TLC on silica gel G. UV spectra were recorded on Systronics double beam UV spectrophotometer 2202, IR spectra were recorded in KBr on Jasco FTIR 4100 spectrophotometer, NMR spectra on Bruker avance II-400 MHz, spectrometer using TMS as internal reference. The student's paired *t*-test (two tailed) was used to analyse significant differences between the control and experimental groups by using the GraphPad Prism version 5.0 for Windows (GraphPad Software, Inc., 2009). Data are expressed as mean ± SEM.

2.1. Defatting of coarsely powdered bark

The bark was dried at room temperature and coarsely powdered. The fresh air-dried and coarsely powdered bark of *Mangifera indica* was extracted exhaustively with petroleum ether (60-80 ℃) in Soxhlet apparatus to remove fatty matter for 56 h.

2.2. Extraction of mangiferin

Coarsely powdered bark of *Mangifera indica* was extracted exhaustively with ethanol (95%) in Soxhlet apparatus for 56 h. The combined alcohol extracts were concentrated under reduced pressure. Then, yellow amorphous powder was obtained.

2.3. Isolation of mangiferin

The dried alcoholic extract was adsorbed on silica gel (60-120 mesh) and chromatographed over silica gel column packed in petroleum ether (60-80 ℃). The column was eluted with chloroform : methanol (1 : 1) which gave mangiferin as a pale yellow amorphous powder. This upon crystallization from ethanol, produced pale yellow needle shaped mangiferin crystals.

2.4. General method for the preparation of mangiferin analogues

A mixture of equal mol of mangiferin, powdered paraformaldehyde and aromatic amine, 10 mL of 95% ethanol and 1 mL of concentrated hydrochloric acid was refluxed, cooled to room temperature and kept in a refrigerator overnight. The solid was filtered and washed with water and recrystallized from ethanol (Figure 1).

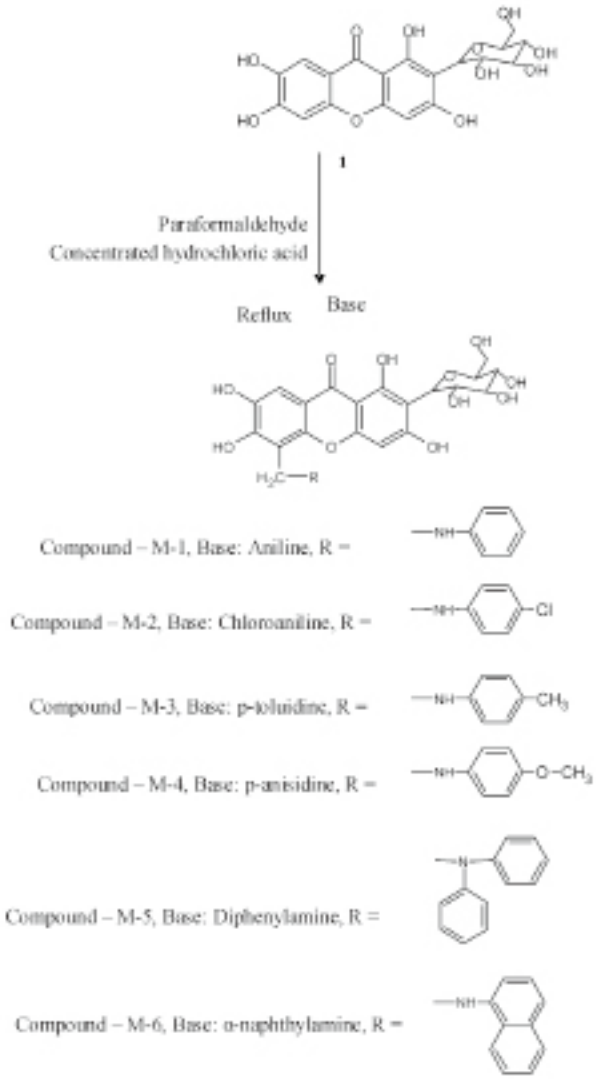

Figure 1. Synthesis of mangiferin analogues.

2.5. Evaluation of antipyretic activity

Swiss albino mice were obtained from Central Animal House, Banaras Hindu University, Reg. No. 542/02/ab/CPCSEA, weighing 25-30 g were given free access to semi synthetic balanced diet and water *ad-libitum*, with occasional supply of green vegetables (salad leaves). Mice were caged four per Perspex experimental cages at room temperature (22-25 ℃). Twelve hours of light and dark cycles were strictly followed in a fully ventilated room.

The method described by Bisigano *et al*[11] was used. The animals were divided into sixteen groups of five animals each (n=5). Pyrexia was induced by subcutaneous injection of 20 mL/kg of 20% w/v freshly prepared aqueous suspension of Brewer's yeast (BY) in normal saline (0.9% w/v NaCl) to mice below the nape of the neck. Mangiferin and analogues (100 mg/kg and 200 mg/kg), acetylsalicylic acid (150 mg/kg) and equivalent volume of distilled water administered *i.p.* to mice in different groups of five animals. The rectal temperature was recorded using a rectal thermometer before beginning the experiment and immediately before and 1, 2 and 3 h after drug administration.

3. Results

3.1. Characterization of mangiferin

Melting point: 269-270 ℃, R_f: 0.77 using n-butanol: acetic acid:water (4∶1∶2.2) as a Solvent system, λ_{max}: 205.6, 256.8, 238.4, 315.2, 367.2 nm. IR (KBr) cm^{-1}: 3 366(O-H), 2 937(C-H), 1 649(>C=O), 1 495(C=C), 1 253(-C-O), 1 050(C-O-C). NMR (δ ppm): 13.81(ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.82 (Ar-H, 1H), 6.36 (Ar-H, 1H), 7.4 (ArH, 1H), 2.5 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 2H), 3.5 (-CH-, 3H).

3.1.1. 5-(N-phenylamino methyleno) mangiferin (M-1)

Melting point: 190 ℃, R_f: 0.60, λ_{max}: 239.6, 261.2, 317.6, 370.4 nm. IR (KBr) cm^{-1}: 3 551(O-H), 3 319(N-H), 2 929(C-H), 1 625(>C=O), 1 488(C=C), 1 383(-C-N), 1 293(-C-O), 1 037(C-O-C). NMR (δ ppm): 13.70 (ArOH intramolecularly bonded, 1H), 8 (ArOH, 3H), 6.82 (Ar-H, 6H), 7.39 (Ar-H, 1H), 3.7 (Ar-CH_2-N-, 2H), 4.1 (Ar-NH-, 1H), 2.9 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.4 (-CH-, 1H), 3.5 (-CH-, 4H).

3.1.2. 5-(N-p-chlorophenylamino methyleno) mangiferin (M-2)

Melting point: 210 ℃, R_f: 0.69, λ_{max}: 225.2, 228.8, 261.2, 318.8, 368 nm. IR (KBr) cm-1: 3 410(O-H), 3 360(N-H), 2 926(C-H), 1 625(>C=O), 1 429(C=C), 1 375(-C-N), 1 295(-C-O), 1 079(C-O-C), 715(C-Cl). NMR (δ ppm): 13.66 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.82 (Ar-H, 5H), 7.36 (Ar-H, 1H), 4.2 (Ar-CH_2-N-, 2H), 4.0 (Ar-NH-, 1H), 2.1 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.4 (-CH-, 5H).

3.1.3. 5-(N-4-methyl phenylamino methyleno) mangiferin (M-3)

Melting point: 195 ℃, R_f: 0.53, λ_{max}: 230, 261.2, 317.6, 370.4 nm. IR (KBr) cm^{-1}: 3 493(O-H), 3 483(N-H), 2 971(C-H), 1 638 (>C=O), 1 429(C=C), 1 283(-C-N), 1 044(C-O-C), 713. NMR (δ ppm): 13.66 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.82 (Ar-H, 5H), 7.36 (Ar-H, 1H), 2.3 (Ar-CH_3, 3H), 3.7 (Ar-CH_2-N-, 2H), 4.2 (Ar-NH-, 1H), 2.3 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 5H).

3.1.4. 5-(N-p-Methoxy phenylamino methyleno) mangiferin (M-4)

Melting point: 190 ℃, R_f: 0.45, λ_{max}: 210.8, 224, 261.2, 317.6, 370.4 nm. IR (KBr) cm^{-1}: 3 536(O-H), 3 445(N-H), 2 941(C-H), 1 646(>C=O), 1 432(C=C), 1 283(-C-N), 1 180(Ar-O-C), 1 078(C-O-C). NMR (δ ppm): 13.66 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.8 (Ar-H, 1H), 6.9 (Ar-H, 4H), 7.36 (Ar-H, 1H), 4.2 (Ar-CH_2-N-, 2H), 4.0 (Ar-NH, 1H), 3.8(Ar-O-CH_3, 3H), 2.1 (-C-OH, 4H), 3.8 (-CH-O-, 2H), 3.3 (-CH-, 5H).

3.1.5. 5-(N, N-Diphenylamino methyleno) mangiferin (M-5)

Melting point: 210 ℃, R_f: 0.82, λ_{max}: 257.6, 240.8, 305.6, 364.4 nm. IR (KBr) cm^{-1}: 3 371(O-H), 2 931(C-H), 1 647(>C=O), 1 405(C=C), 1 297(-C-N), 1 253(-C-O), 1 031(C-O-C). NMR (δ ppm): 13.78 (ArOH intramolecularly bonded, 1H), 7.87 (ArOH, 3H), 6.84 (Ar-H, 2H), 7.4 (Ar-H, 2H), 7.04 (Ar-H, 4H), 7.02 (Ar-H, 4H), 3.9 (Ar-CH_2-N-, 2H), 2.1 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 2H), 3.4 (-CH-, 3H).

3.1.6. 5-(N- α -Napthylamino methyleno)-mangiferin (M-6)

Melting point: 205 ℃, R_f: 0.60, λ_{max}: 244.4, 297.2, 306.8nm. IR (KBr) cm^{-1}: 3 443(O-H), 3 339(N-H), 2 927(C-H), 1 621(>C=O), 1 482(C=C), 1 385(-C-N), 1 290(-C-O), 1 038(C-O-C). NMR (δ ppm): 13.78 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.87 (Ar-H, 1H), 7.36 (Ar-H, 1H), 7.4 (napth-H, 5H), 7.5 (napth-H, 2H), 4.29 (Ar-CH_2-N-, 2H), 4.1(Ar-NH-, 1H), 2.1 (-C-OH, 4H), 3.8 (-CH_2-O-, 2H), 3.4 (-CH-, 5H).

3.2. Evaluation of antipyretic activity

Table 1 showed that all the analogues of mangiferin along with acetyl salicylic acid (ASA) showed a significant reduction in rectal temperature up to 3 h. At initial stage *ie.* up to 1st h ASA, mangiferin, M-5 and M-6 showed a highly significant activity at both dose level *ie.*100 and 200 mg/kg ($P< 0.001$), whereas in case of other analogues the same effect was observed only at 200 mg/kg. Further, at the end of 2nd phase all the analogues showed a significant effect

Table 1
Antipyretic activity of mangiferin analogues in mice (℃).

Treatment	Before treatment	After treatment			
		0 h	1 h	2 h	3 h
Control	38.76±0.05	38.65±0.05	38.76±0.05	38.60±0.04	38.61±0.05
ASA (150 mg)	38.80±0.04	38.70±0.04	38.13±0.02***	37.45±0.12***	36.31±0.01***
MG (100 mg)	38.86±0.03	38.75±0.03	38.13±0.04***	37.38±0.03***	37.07±0.01***
MG (200 mg)	38.86±0.03	38.85±0.03	37.87±0.05***	36.76±0.03***	36.33± 0.02***
M-1 (100 mg)	38.93±0.02	38.81±0.02	38.88±0.01	38.78±0.02***	37.96± 0.03***
M-1 (200 mg)	38.92±0.02	38.82±0.02	38.73±0.02***	38.38±0.15***	37.20±0.02***
M-2 (100 mg)	38.86±0.02	38.75±0.02	38.381±0.01	38.59±0.02	37.68±0.20***
M-2 (200 mg)	38.88±0.02	38.66±0.01	38.59±0.02***	38.36±0.01***	37.23±0.02***
M-3 (100 mg)	38.85±0.03	38.83±0.03	38.54± 0.02*	38.41±0.04*	37.93±0.19***
M-3 (200 mg)	38.89±0.01	38.84±0.01	37.44±0.02***	37.16±0.02***	36.69±0.09***
M-4 (100 mg)	38.90±0.26	38.79±0.24	38.62±0.01	38.56±0.01	37.94±0.05***
M-4 (200 mg)	38.89±0.04	38.77± 0.04	37.88±0.06***	36.98±0.08***	36.53±0.28***
M-5 (100 mg)	38.89±0.02	38.77±0.01	38.59±0.02**	37.99±0.08***	37.79± 0.10***
M-5 (200 mg)	38.82±0.03	38.62±0.03	37.92±0.07***	37.53±0.11***	36.51±0.04***
M-6 (100 mg)	38.87±0.03	38.86± 0.02	38.58±0.04***	38.29±0.03***	37.94± 0.02***
M-6 (200 mg)	38.66±0.02	38.65± 0.02	37.90±0.08***	37.31±0.09***	36.73± 0.04***

*indicates $P< 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$ compared to temperature before treatment.

at both dose level ($P< 0.001$) except for M-3 (100 mg/kg) which was found to be less significant ($P< 0.05$) while M-2 (100 mg) and M-4 (100 mg) were not found to be significant. At the end of 3rd phase all the analogue showed a significant reduction in the rectal temperature ($P< 0.001$).

4. Discussion

In the process of isolation of mangiferin, stem bark of *Mangifera indica* was defatted with petroleum ether (60-80 ℃) prior to extraction with ethanol 95%. The extract was chromatographed over silica gel and eluted with chloroform: methanol (1 : 1) to afford the parent mangiferin as pale yellow needle shaped crystals. Mangiferin analogues such as 5-(N-phenylamino methyleno) mangiferin, 5-(N-p-chlorophenylamino methyleno) mangiferin, 5-(N-4-methyl phenylamino methyleno) mangiferin, 5-(N-p-methoxy phenylamino methyleno) mangiferin, 5-(N, N-diphenylamino methyleno) mangiferin, 5-(N- α -napthylamino methyleno) mangiferin were synthesized. The synthesized mangiferin analogues were characterized by R_f, mp, UV, IR and NMR spectral analyses. The absorbed maxima 205.6, 256.8, 238.4, 315.2 and 367.2 nm of mangiferin is closely related to that of reported UV spectral data[12]. Mangiferin and its derivative were also confirmed by proton NMR signals. Subcutaneous injection of BY markedly increase the mice rectal temperature. Elevation in body temperature in response to an invasive or infectious agent is known as fever. Invasive agents like BY are known to stimulate synthesis of various cytokines (TNF- α , IL-1, IL-6). The pyretic effect of BY was maintained for more than 3 hr after drug or vehicle treatment. Mangiferin has been previously reported to have anti-inflammatory properties. Recent study has shown that mangiferin inhibits TNF- α synthesis. Mangiferin also has been reported to possess anti-oxidant activity which is useful for amelioration of inflammatory actions of cytokines. Similar results were observed in the previous study. All the analogues of mangiferin in both the doses (100 mg/kg and 200 mg/kg) as well as ASA had significantly decreased the rectal temperature up to 3 hr. Lower doses (100 mg/kg) of M-1, M-2 and M-4 were not able to reduce the rectal temperature within 1 h. M-1 (100 mg/kg) and M-4 (100 mg/kg) had significantly decreased temperature after 2 h, whereas M-2 (100 mg/kg) had shown anti pyretic effect after 3 h. Higher doses of analogues were more effective in decreasing the rectal temperature than lower doses. All the analogues in higher doses had shown anti pyretic activity at 1 h and the activity was sustained up to 3 h. These observations of present study reveal that the mangiferin and mangiferin analogues have antipyretic activity.

From the present study it can be concluded that the synthesized analogue along with mangiferin showed a significant antipyretic activity. The activity may be due to the anti-inflammatory and antioxidant potential of mangiferin or its analogues. However, further investigations are required to know the exact mechanism of action which may prove it to be a potential antipyretic agent.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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