Acknowledgements

This work was supported by a grant from the joint research fund of the Hebrew University and Hadassah.

References

(1) Lu, A. Y. H., West, S. B. (1978) Pharmac. Ther. 2, 337–358.
(2) Alvares, A. P. (1981) Drug Met. Rev. 12, 431–436.
(3) Park, B. K. (1982) Br. J. Clin. Pharmac. 14, 631–651.
(4) Groves, J. T., Nemo, T. E., Myers, R. S. (1979) J. Am. Chem. Soc. 101, 1032–1033.
(5) Peterson, J. A., Ishimura, Y., Baron, J., Estabrook, R. W. (1973) Oxidases and Related Redox Systems, p. 565, University Park Press, Baltimore.
(6) Rahimtula, A. D., O'Brien, P. J., Hrycay, E. G., Peterson, J. A., Estabrook, R. W. (1974) Biochem. Biophys. Res. Commun. 60, 695–702.
(7) Lightenberger, F., Nastainczyk, W., Ullrich, V. (1976) Biochem. Biophys. Res. Commun. 70, 939–946.
(8) Van der Hoeven, J. A., Coon, M. J. (1974) J. Biol. Chem. 249, 6302–6310.
(9) Fahl, W. E., Nesnow, S., Jefcoate, C. R. (1977) Arch. Biochem. Biophys. 181, 649–664.
(10) Omura, T., Sato, R. (1964) J. Biol. Chem. 239, 2379–2385.
(11) Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
(12) Fujii, K., Tomino, K., Watanabe, H. (1955) J. Pharm. Soc. Japan, 74, 1049–1051.
(13) Robson, J. M., Reinhart, J. (1955) J. Am. Chem. Soc. 77, 2453–2457.
(14) De Bennevile, P. L., Strong, J. S., Elkind, V. T. (1956) J. Org. Chem. 21, 772–773.
(15) Nash, T. (1953) Biochem. J. 55, 416–421.
(16) Cochin, J., Axelrod, J. (1959) J. Pharmacol. Exp. Ther. 125, 105–110.
(17) Gillette, J. R., Davis, D. C., Sasame, H. A. (1972) Ann. Rev. Pharmacol. 12, 57–84.
(18) Jefcoate, C. R. (1978) Methods in Enzymology, Vol. LII, Part C, pp. 258–279. Academic Press, New York.
(19) Groves, J. T., Van Der Puy, M. (1976) J. Am. Chem. Soc. 98, 5290–5297.

Effect of Mangiferin, a Naturally Occurring Glucosylxanthone, on Reproductive Function of Rats

Sukumar Chattopadhyay[1], Utpala Chattopadhyay[1], Santyeswar P. Shukla[2], and Shibnath Ghosal[2,3]

Received: February 7, 1984; accepted: July 20, 1984.

Abstract: Female albino rats, treated with mangiferin at daily doses of 5 mg/100 g ip for 3 days during mid-gestation, showed complete fetal resorption. Administration of a mangiferin-Cu^{2+} (1:1) complex, along with mangiferin, attenuated the effect of mangiferin and restored the completion of gestation in 60% of the rats. The mother rats that completed gestation successfully did not show any post-natal abnormality, and the litters born were also normal. Mangiferin treatment of non-gestating female rats caused little or no changes in their organ weights. However, changes in the protein and protein-DNA ratios of several organs were statistically significant. Mangiferin also modified the ascorbic acid retention capacity of adrenal glands of male rats *in vitro*. These findings are appraised in view of mangiferin as a potential antifertility agent.

[1] Department of Physiology, Institute of Medical Sciences, Banaras Hindu University
[2] Department of Pharmaceutics, Institute of Technology, Banaras Hindu University
[3] Correspondence address Prof. Ghosal, Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-221005, India

Mangiferin (1,3,6,7-tetrahydroxyxanthone-C_2-β-D-glucoside) is widely distributed in higher plants. The glucosylxanthone was isolated in substantial amounts from *Canscora decussata* Schult (family Gentianaceae) (1), *Swertia chirata* Buch.-Ham. (Gentianaceae) (2), and *Mangifera indica* Linn. (Anacardiaceae) (3), following their reputation as valuable plant drugs in the Indian systems of medicine. The current study is part of a program to evaluate the potential of phytochemicals as antifertility agents (4, 5). It was prompted by the following observations. (i) Ingestion of tender leaves of *M. indica*, which contain 2% mangiferin (3), caused 'anti-spring flush' (decrease in yield of milk) in dairy cattle (5). Chemical agents which inhibit prolactin synthesis (cause of 'anti-spring flush') have been found to also interrupt pregnancy (6). (ii) Mangiferin was previously found to inhibit monoamine oxidase in the CNS of albino mice and rats (2, 7–9). Inhibition of pregnancy in mammals by naturally occurring CNS active agents is a well documented phenomenon (10). Thus, an antifertility effect of mangiferin was considered a reasonable possibility.

Materials and Methods

Mangiferin was isolated from tender leaves of *Mangifera indica* Linn. (cv Banarasi Langra) according to a published procedure (3).

Animals

Rats of the CF strain were bred and maintained, under controlled conditions (12 h light, 22 ± 3° C), with Hindusthan Lever food pellets and tap water ad libitum. Female rats (150–200 g) with regular estrus cycle were mated with male rats of proven fertility. Presence of sperms in vaginal smear of female rats, in estrus, was taken as day 1 of pregnancy. Pregnancy was confirmed by laparotomy on parallel groups of rats.

Gestation

Female rats showing presence of sperms in the vaginal smear were given mangiferin, suspended in saline, intraperitoneally (ip) at doses of 1.0, 2.5 and 5 mg/100 g body weight (b.w.) day^{-1}, for 3 days at mid-gestation, i. e. on day 12, 13, and 14. The rats were sacrificed along with those treated with the vehicle (saline) on day 20 of gestation, and the uteri were examined for the presence of normal fetus or signs of resorption. Distinct areas of hemorrhage and sign of tissue debris were taken as points of resorption. A total dose of 15 mg of

mangiferin was found to be effective and was tested on different periods of gestation in the following manner (a–c).

(a) Mangiferin (5 mg/100 g b.w. ip day^{-1}), in saline suspension, was administered on day (i) 1, 2, 3 of gestation; (ii) 12, 13, 14 of gestation; (iii) 18, 19, 20 of gestation.

(b) Mangiferin (10 mg/100 g b.w. sc day^{-1}), in aqueous propylene glycol (10 %), was administered on day (i) 12 of gestation; (ii) 19 of gestation.

(c) Mangiferin-Cu^{2+} complex (5 mg/100 g b.w. sc day^{-1}) + mangiferin (5 mg/100 g b.w. sc day^{-1}), in aqueous propylene glycol (10 %), were administered on day 12, 13, 14 of gestation.

Organs

Organ weights of liver, kidneys, adrenals, uterus, and ovaries were determined on day 20 of commencement of treatment with the test compounds. Saline only was used in the control group. The cleaned organs were weighed, and DNA and protein were determined according to published procedures (11).

Adrenal Gland Incubation

The adrenal glands were collected from normal male rats following cervical crush, cleaned of adhering tissues, quartered, and incubated in Krebs-Ringer bicarbonate medium at 37° C with 95 % O_2 and 5 % CO_2 to maintain the pH at 7.4. In each experiment (control and mangiferin-treated), 4 rats were used, and the quarters of each adrenal were placed in each of 4 flat-bottom plastic vials containing 4 ml fluid. A solution of mangiferin (0.1 ml) (40 μg mangiferin/100 ml saline), was added to each vial. After incubation for 2 h the supernatant was decanted on a moist filter paper, and the tissues were collected in chilled metaphosphoric acid (2.5 %, 5 ml). The supernatants of the washed and chilled homogenized tissues were collected by centrifugation (10,000 g for 15 min, at 4°C) and assayed by the color reaction of 2,6-dichlorophenol indophenol for ascorbic acid. In the control group, only saline was used (in place of mangiferin).

The statistical significance of the data (Tables I–V) was analyzed according to published procedures (12).

Mangiferin-Cu^{2+} Complex

Mangiferin (0.418 g) in aqueous ethyl alcohol (50 %, 50 ml) and cupric acetate (0.208 g) dissolved in the same solvent were gradually mixed and kept at room temperature overnight. The resultant yellow precipitate was collected by filtration, washed with water and diethyl ether in succession and dried in vacuo (over P_2O_5). The solid (35 mg) did not melt up to 300°C but decomposed at this temperature; UV: $\lambda_{max}^{ethyl\ alcohol}$ 242, ~275, ~340 nm; IR: ν_{max}^{Nujol} 3380–3350, 1600 (broad), 1020 (broad) cm^{-1} (while mangiferin exhibited major bands at ν3380, 1648, 1612, 1595, 1040, 1025 cm^{-1}). The ratio of mangiferin: Cu in the complex was determined after acidification with dilute HCl and extraction of the liberated mangiferin with *n*-BuOH. Further workup (3) provided mangiferin as an amorphous solid, that was found to be homogeneous by TLC and was assayed by UV spectrometry (1, 3). The amount of Cu^{2+} in the incinerated residue obtained from the extracted aqueous phase was determined (as the chloride) by atomic absorption spectroscopy (Perkin-Elmer model 373). Thus, in a 13.06 mg of the mangiferin-Cu^{2+} complex, the amounts of mangiferin and Cu^{2+} were 11.55 and 1.51 mg, respectively (n=5), which corresponds to 1:1 molar ratio.

The formation of mangiferin-Cu^{2+} complex in ethyl alcohol was monitored with repetitive UV scans by adding a 0.1 mM cupric acetate solution into the cuvette of a Beckmann ratio-recording spectrophotometer (model 524), containing a 0.1 mM solution of mangiferin until 1:1 stoichiometry was attained.

Results and Discussion

Administration of mangiferin (5 mg/100 g b.w. ip day^{-1} for 3 days) to female albino rats, at mid-gestation, caused 100 % resorption of the fetus (Table I). Smaller doses and number of administration of mangiferin, or administration of mangiferin at other periods of gestation caused fetal resorption in varying proportions (Tables I and II). Treatment with a mangiferin-Cu^{2+} complex (5) (5 mg/100 g b.w. ip day^{-1} for 3 days) along with the same dose of mangiferin, during mid-gestation, attenuated the fetal resorption effect of mangiferin, and complete gestation was noted in 60 % of the animals (Table II). Additionally, mangiferin caused some changes in the organ weights of female rats. The results were, however, statistically insignificant (Table III). The changes in the concentration of protein and protein-DNA ratios in some organs, in response to mangiferin treatment, were statistically significant (Table IV). Mangiferin was also found to cause retention of ascorbic acid in adrenal glands of male rats when tested *in vitro* (Table V).

The rats that completed gestation did not show any deviation from the normal pattern of gestation and in post-natal behavior. The litters born did not bear any physiological or teratological abnormality.

Although the mechanism of fetal resorption due to mangiferin has not been entirely elucidated, the following observations would seem to be relevant. Interaction of mangiferin with Cu^{2+} ions *in vitro*, and the effect of mangiferin-Cu^{2+} complex on the gestation of albino rats were examined, since fetal resorption in nonprimates is often associated with deficiency/impairment of essential trace elements, eg. Cu (13). In an earlier study on the etiology of malformation disease of *M. indica*, a close connection between the disease syndromes and accumulation of mangiferin, with concomitant depletion of Cu^{2+} and Zn^{2+} ions in the diseased parts of the plant was discerned (14). A normal copper and zinc balance could be restored to the diseased parts by spraying with mangiferin-metal chelates, which led to the emergence of healthy twigs (5). In the present study, the effect of mangiferin-Cu^{2+} complex on completing the gestation of albino rats also suggested a significant role of the mangiferin-copper interaction during fetal development.

The decrease in the concentration of liver proteins by mangiferin could also be due to an impaired function of Cu^{2+} ions, since in the liver of adult rats protein synthesis is inducible by copper ions (15). This hypothesis is supported by the formation of a stable mangiferin-Cu^{2+} complex in solution. The sequence of formation of the mangiferin-Cu^{2+} complex was monitored by UV spectrophotometry. While the absorption maximum of the mangiferin-Cu^{2+} complex at 240 nm was similar to that of the mangiferin band-II (1), complex formation with Cu^{2+} resulted in a considerable increase in the intensity (hyperchromic effect) of this maximum until a 1:1 stoichiometry of the mangiferin-Cu^{2+}complex was attained. These observations together with further spectral changes indicated the formation of a mangiferin-Cu^{2+} complex of the donor-acceptor type (16).

Mangiferin produced a slight increase in the weights of liver, adrenals, and

Table I. Effect of Mangiferin on the Gestation of Female Rats at Mid-gestation

Group[a]	Dose[b] mg/100 g ip day^{-1} for 3 days	percent completed gestation	Mean sites of fetal resorption	P
1. Mangiferin	1.0	80	3.0	
2. Mangiferin	2.5	60	4.5	
3. Mangiferin	5.0	nil	3.6	<0.05[c]
4. Control	–	90	nil	

[a]Ten animals in each experiment. [b]In saline on day 12, 13, 14 of gestation.
[c]Significance in relation to group 4.

Table II. Effect of Mangiferin and Mangiferin-Cu^{2+} Complex on the Gestation of Female Rats (drugs applied at different periods of gestation)

Group[a]	Dose mg/100 g day^{-1}	Day of gestation	Percent of completed gestation	P
1. Mangiferin[b]	5.0	1, 2, 3	70	
2. Mangiferin[b]	5.0	12, 13, 14	nil	<0.05[d]
3. Mangiferin[b]	5.0	18, 19, 20	80	
4. Mangiferin[c]	10.0	12	30	
5. Mangiferin[c]	10.0	19	80	
6. Mangiferin[c] + mangiferin-Cu complex	5 + 5	12, 13, 14	60	< 0.05[e]
7. control	–	12, 13, 14	90	

[a]Ten animals in each experiment. [b]In saline (ip). [c]in propylene glycol (10%, sc.).
[d]Significance in relation to group 7. [e]significance in relation to group 2.

Table III. Effect of Mangiferin and Mangiferin-Cu^{2+} Complex on Organ Weights of Female Rats

group[a]	liver g	kidney g	adrenal mg	uterus g	overy mg
mangiferin[b]	3.6±0.17	0.72±0.06	23.8±1.7	2.76±1.90	24.3±7.5
mangiferin + mangiferin-Cu complex	3.2±0.23	0.76±0.06	28.1±3.2	1.98±0.60	27.8±7.8
control	3.2±0.16	0.76±0.05	21.8±2.4	2.02±0.81	28.5±7.0

[a]Six animals in each experiment; values represent mean ± standard deviation (s.d.).
[b]In saline (5 mg/100 g b.w. ip day^{-1} for 5 days).
[c]In saline (5 mg/100 g b.w. sc day^{-1} for 5 days). The data were statistically insignificant in comparison to those of the control.

Table IV. Effect of Mangiferin on the DNA and Protein Concentrations of Liver, Uterus and Ovary of Female Rats

Group[a]	liver		uterus		ovary	
	protein mg/100 mg	DNA mg/g	protein mg/100 mg	DNA mg/g	protein mg/100 mg	DNA mg/g
mangiferin[b]	13.87±0.5	2.96±0.9	9.39±0.42	2.58±0.5	8.66±1.0	2.87±0.5
control	16.42±0.7	5.78±1.5	8.15±0.3	2.81±0.6	8.46±0.3	2.89±0.5
P	<0.05	<0.05	<0.05	n.s.	n.s.	n.s

[a]Six animals in each experiment; values represent mean ± s.d.
[b]In saline (5 mg/100 g ip day^{-1} for 5 days). n.s., not significant.

Table V. Effect of Mangiferin *in vitro* on the Ascorbic Acid Content of Adrenal Gland of Male Rats

Group[a]	Ascorbic Acid (μg/100 mg[c])
Mangiferin[b]	180±19
control	108±27

[a]four animals were taken; values represent mean ± s.d.
[b]in saline (ca. 2.5 x 10^{-8} M/vial)
[c]fresh adrenal weight

uterus of rats over those of the control. The weights of kidney and ovary, however, showed a slight decrease (Table III). Treatment of mangiferin also produced a significant decrease in the concentrations of protein and DNA in the liver but an increase in the protein/DNA ratio (Table IV). These observations suggest that mangiferin causes a metabolic change in the liver leading, possibly, to an increase in cell volume. This increase in cell volume might have occurred as a physiological hypertrophy in response to an antimitotic effect of mangiferin (17). The protein/DNA ratio in the uterus also showed an increase. The effect of mangiferin on the organ weights was reversed when mangiferin-Cu^{2+} complex was administered along with mangiferin. The values were closely similar to those of the control (Table III).

The effect of mangiferin on the retention of ascorbic acid in the adrenal gland *in vitro* (Table V) suggests that mangiferin is competing with ascorbic acid for binding with the essential trace elements (eg. Cu^{2+}). It might also have contributed to the transport of such trace elements.

In the absence of any abnormality in mother rats and also in the surviving litters, on treatment with mangiferin, at any stage of gestation, it seems likely that the fetal resorption is not due to any toxicity of the test compound. Further examination of the above aspects would help to establish as to how mangiferin inhibits fetal growth and causes fetal resorption in albino rats. Studies to evaluate the potential of mangiferin as an antifertility agent are currently in progress.

Acknowledgements

We thank the National Cancer Institute, Bethesda, USA, for antimitotic screening

data of mangiferin. S.P.S. thanks the University Grants Commission, New Delhi, India, for a research fellowship.

References

(1) Chaudhuri, R. K., Ghosal, S. (1971) Phytochemistry 10, 2425–2432.
(2) Ghosal, S., Sharma, P. V., Chaudhuri, R. K., Bhattacharya, S. K. (1973) J. Pharm. Sci. 62, 926–930.
(3) Ghosal, S., Biswas, K., Chattopadhyay, B. K. (1978) Phytochemistry 17, 689–694.
(4) Ghosal, S., Srivastava, A. K., Srivastava, R. S., Chattopadhyay, S., Maitra, M. (1981) Planta Med. 42, 279–283.
(5) Shukla, S. P. (Ghosal, S., Supervisor) (1981) M. Pharm. thesis, pp. 22–43, Banaras Hindu University, Varanasi.
(6) Chattopadhyay, S., Chattopadhyay, U., Mathur, P. P., Saini, K. S., Ghosal, S. (1983) Planta Med. 49, 252–254.
(7) Bhattacharya, S. K., Ghosal, S., Chaudhuri, R. K., Sanyal, A. K. (1972) J. Pharm. Sci. 61, 1838–1840.
(8) Bhattacharya, S. K., Sanyal, A. K., Ghosal, S. (1972) Naturwissenschaften 59, 651.
(9) Bhattacharya, S. K., Sanyal, A. K., Ghosal, S. (1976) in Drugs and Central Synaptic Transmission (Bradley, P. B., Dhawan, B. N., eds.) pp. 333–340, Macmillan, London.
(10) Farnsworth, N. R., Bingel, A. S., Cordell, G. A., Crane, F. A., Fong, H. H. S. (1975) J. Pharm. Sci. 64, 535–598.
(11) Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193, 265–282.
(12) Lembeck, F., Sewing, K.-Fr. (1966) in Pharmacological Facts and Figures, pp. 104–109, The English University Press, London.
(13) Hurley, L. S., Keen, C. L., Lonnerdal, B. (1980) in Excerpta Medica, pp. 227–272, Ciba Foundation Symposium 79, New York.
(14) Ghosal, S., Chakrabarti, D. K., Biswas, K., Kumar, Y. (1979) Experientia 35, 1633–1634.
(15) Evans, G. W., Johnson, P. E. (1978) Arbeit. f. Tier. Freising-Weihenstephan 3, 98–99.
(16) Ghosal, S., Dutta, S. K. (1968) Indian J. Chem. 6, 301–306.
(17) Ghosal, S. (1976) in Recent Advances in the Chemistry and Pharmacology of Indian Plant Drugs, pp. 1–21, Andhra University Press, Waltair, India.