Analgesic and Antioxidant Activity of Mangiferin and Its Derivatives: the Structure Activity Relationship

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Mangiferin, 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9*H*-xanthen-9-one, obtained directly from methanolic extracts of *Bombax ceiba* leaves in substantial amounts demonstrated strong antioxidant activity (EC_{50} 5.8±0.96 μg/ml or 13.74 μM) using DPPH assay comparable to rutin, commonly used as antioxidant for medical purposes. The acetyl and cinnamoyl derivatives were found to be less active than mangiferin whereas, methyl and 3,6,7-trimethylether tetraacetate derivatives were inactive implying that for antioxidant activity, free hydroxyl groups and catechol moiety are essential. Moreover, mangiferin showed hepatoprotective activity against carbon tetrachloride induced liver injury further supporting the free radical scavenging property in the *in vivo* system. Additionally, plant extracts and mangiferin failed to exhibit acute anti-inflammatory activity whereas, it displayed significant analgesic effect in acetic acid-induced writhing and hot plate tests in mice. Using naloxone, it was revealed that plant extracts induced analgesia was independent of opioid receptor, whereas, mangiferin demonstrated significant interaction with it at peripheral site with a slight contribution at the neuronal level.

Key words *Bombax ceiba*; mangiferin; derivative; antioxidant; analgesic; hepatoprotective

Bombax ceiba L. (syn. *B. malabaricum* DC), commonly known as Simul, Simbal or Silk-cotton tree belongs to the family Bombacaceae. It is reputed as an important medicinal plant.[1,2] Phytochemical studies on various parts of *B. ceiba* revealed that it is rich in phenolic compounds. While mangiferin,[3] a xanthone, is present in large amounts in the leaves, and obtained directly from the extract.[4] It has significant effect as a hypotensive agent and possesses hypoglycemic activity with negligible toxicity.[4—6]

There is a considerable interest in the antioxidant and free radical scavanging properties of medicinal plants and compounds isolated from them[7] particularly polyphenols which are a broad family of naturally occurring physiologically active nutrients. Xanthones closely related to the polyphenol family, have a remarkable effect on cardiovascular system, are antibiotic, antiviral, anti-inflammatory and also includes some of the most powerful natural antioxidants.[8] It is desirable to search for natural products that can be used to prevent the free radical initiation or the progression of a number of diseases. Free radical damages can be considered as one of the major causative factor involved in many diseases, including inflammation and dementia. It is well established that antioxidants prevent injury to blood vessel membranes, thereby optimizing blood flow to the heart and brain, defend against cancer-causing DNA damages and thus help lowering the risk of cancer, cardiovascular and various mental illnesses including Alzheimer's diseases.[9]

It is well established that reactive oxygen species such as O_2^- and OH play a prominent role in the stimulation, propagation and maintenance of both acute and chronic inflammatory processes as well as pain causing tissue damage. These adverse effects due to pain and excessive inflammation has been shown to be reduced by the use of suitable antioxidants either by preventing the formation of oxygen free radicals or by scavenging them before they react with sites such as unsaturated lipids in the cell membrane.[10,11]

Various parts of *B. ceiba* have been claimed to cure chronic inflammation without any pharmacological studies,[12] therefore it was subjected to anti-inflammatory and analgesic testing. The present investigation on the extracts of leaves of the plant were subjected to bioassay monitored fractionation[4] which resulted in the isolation of mangiferin in substantial amounts possessing promising antioxidant and analgesic activities.

MATERIALS AND METHODS

Isolation of Mangiferin (1) Fresh, uncrushed, undried leaves (3 kg) of *B. ceiba* were percolated at room temperature with methanol for three times, the combined methanolic extracts were freed of the solvent *in vacuo* to a thickish mass (BCL). On keeping overnight a solid matter separated out, it was filtered and washed with hot ethylacetate, methanol and water, affording pure light yellow crystalline powder of mangiferin (**1**) (BCP), 28 g (0.933%) and the filtrate (BCM).[4]

Acetylation of Mangiferin (1) Solution of mangiferin (**1**) (50 mg) in pyridine (4 ml) was treated with acetic anhydride (3 ml) and left for 36 h at room temperature. Reaction mixture on evaporation gave a residue which was divided into chloroform soluble (BCP-AC, **1a**) and insoluble fractions. The spectral studies identified the structure of **1a** as 2-β-D-tetraacetoxyglucopyranosyl-1,3,6,7-tetraacetoxy-9*H*-xanthen-9-one.[13]

Cinnamoylation of Mangiferin (1) Mangiferin (100 mg) was subjected to cinnamoylation with cinnamoyl chloride (10 mg) in presence of pyridine (6 ml) at room temperature for twenty hours. The reaction mixture on evaporation gave a residue, which on fractionation through solvent–solvent separation [petroleum ether, chloroform and methanol, in order of increasing polarity] gave thirty fractions. Fraction 24 ($CHCl_3$: MeOH, 9 : 1) having cinnamoyl derivative and pyridinium salt was treated with ethyl acetate (E.A) and water (H_2O) and the two layers were separated. The E.A phase

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showed a single spot on TLC [silica gel, E.A : acetone : formic acid : H_2O (8 : 2 : 1 : 1), *Rf*=0.55] and was characterized as 2-β-D-glucopyranosyl-7-cinnamoyloxy-1,3,6-trihydroxy-9*H*-xanthen-9-one (**3**) by spectral studies including UV, IR, MS and ^{1}H-NMR experiments. It is a new compound.

Methylation of Mangiferin (1) A solution of mangiferin (500 mg) in acetone (125 ml) was treated with dimethyl sulphate (DMS) (0.855 mg) and K_2CO_3 (100 mg) with stirring at room temperature for seven days. The reaction mixture on evaporation under hood gave a residue, that on solvent–solvent separation (petroleum ether, chloroform and methanol, in order of increasing polarity) afforded thirteen fractions. Fraction 8 ($CHCl_3$: MeOH, 8 : 2) showed single spot on TLC [silica gel, E.A : MeOH : H_2O (8 : 2 : 1), *Rf*=0.5]. Its structure was elucidated as 2-β-D-tetrahydroxyglucopyranosyl-3,6,7-trimethoxy-1-hydroxy-9*H*-xanthen-9-one (**2**).[14,15]

Acetylation of Compound 2 Compound **2** (10 mg) was treated with acetic anhydride (1 ml) at room temperature for seven hours. Reaction product on evaporation gave a residue, which showed a single spot on TLC [silica gel, $CHCl_3$: MeOH (9.9 : 0.1), *Rf*=0.7]. Spectral studies of the compound, characterized its structure as 2-β-D-tetraacetoxyglucopyranosyl-3,6,7-trimethoxy-1-hydroxy-9*H*-xanthen-9-one (**2a**).[15]

Animals and Drugs NMRI mice (22—28 g) and Wistar rats (180—200 g) of either sex were obtained from animal house facility of H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan. Animals were housed 10 per cage under standard environmental condition with 12 h light and dark period with free access to food and water. Ethical principles established in 1979 for laboratory animals at the service of mankind Lyons, France were followed.

The chemicals used in this study include: aspirin (Reckitt and Colman, Pakistan), acetic acid, carrageenan, carbon tetrachloride, brain extract Type VII, 1,1-diphenyl-2-picrylhydrazyl (DPPH), deoxyribose, morphine sulphate, naloxone, rutin and all other reagents used were of analytical grade (Sigma chemicals company, St. Louis, U.S.A.).

Free Radical Scavenging Assays. DPPH Free Radical Scavenging Assay This method is based on the scavenging activity of stable DPPH free radicals.[16] Reaction mixture containing test samples 2—100 μg/ml and 300 μM DPPH ethanolic solution were left at room temperature for a period of 30 min. Absorbance was measured at 517 nm on a UV–VIS spectrophotometer (Shimadzu UV-2200) and percent inhibition after sample treatment was calculated. IC_{50} values graphically obtained represented the concentration of sample required to scavenge 50% of free radicals.

Deoxyribose Degradation Assay Hydroxyl radical damage, to the deoxyribose was assayed by the formation of thiobarbituric acid reactive substances, measured spectrophotometrically at 532 nm.[9,17]

Non-enzymatic Lipid Peroxidation in Liposomes Bovine brain extract Type VII (5 mg/ml), ferric chloride and ascorbic acid were tested with different concentration of the compounds to induce OH radical generation. Lipid peroxidation was measured by the thiobarbituric acid (TBA) reaction at 532 nm.[17,18]

Liver Injury Test It was performed as described previously.[19,20] Thirty min prior to 20% carbon tetrachloride (CCl_4, 1.5 ml/kg) treatment animals received either 10% DMSO or olive oil (control) or BCL, BCM (1—100 mg/kg) or mangiferin (0.1, 1, 10 mg/kg) orally. After 24 h the animals were anaesthetized with pentothal sodium (60 mg/kg) and the blood was collected from carotid artery. The blood was centrifuged for 10 min at 5000 rpm to obtain serum that was used to determine aspartate transaminase (AST) and alanine transaminase (ALT) levels by the kits (International diagnostic links Chicago, U.S.A.).

Acetic Acid-Induced Writhing in Mice The writhing test was performed on Albino mice.[21] Animals were pretreated with vehicle (10% DMSO), BCM, BCL (10, 50, 100 mg/kg), mangiferin (**1**) (0.422, 4.22, 42.2 mg/kg), or with standard analgesic drug aspirin intraperitoneally 30 min before 0.9% acetic acid (0.9% acetic acid in saline) was administered intraperitoneally (10 ml/kg). To investigate the mechanism of action, mice were treated with naloxone (5 mg/kg). After 10 min morphine (0.25 mg/kg) or mangiferin (**1**) (42.2 mg/kg) was administered sub-cutaneously followed by administration of 0.6% acetic acid (0.6% acetic acid in saline) intraperitoneally (10 ml/kg) and the number of writhes were noted. The number of writhes in each treated group was compared with control (DMSO treated group) and has been represented as percent inhibition of the writhes.

Hot Plate Test The hot plate was used to estimate the latency of responses as described earlier.[22] Mice were placed on a metal plate heated to a temperature of 50$\pm$0.05 °C. The mice were treated either with vehicle 10% DMSO (10 ml/kg, control) or BCL, BCM (100 mg/kg) and mangiferin (**1**) (42.2 mg/kg) or morphine (10 mg/kg) intraperitoneally, and the response time was noted at 0, 30, 60, 90 and 120 min with a cutoff time of 20—30 s. An opioid non-selective antagonist naloxone (2 mg/kg) was injected 15 min prior to the administration of test sample and observed as explained above. Analgesic activity was expressed as the increase in response time with respect to control.

Strychnine-Induced Lethality in Mice Animals were divided into 2 groups of 10 mice each. One group was given vehicle (10% DMSO; 10 ml/kg; orally) followed after 1 h by oral administration of strychnine (1 mg/kg). The group 2 mice were treated similarly to group 1 except that mangiferin (10 mg/kg; orally) was given instead of vehicle. The animals were observed for mortality occurring within 2 h.

Statistical Analysis The results are represented as mean$\pm$standard error of mean. The difference between control and test group was estimated by one way analysis of variance using Tukey HSD test. The results were considered significantly when $p<0.05$ as compared to control.

RESULTS AND DISCUSSION

The results obtained in the present study demonstrate that methanolic leaf extract of *B. ceiba* and pure compound mangiferin (**1**) have antioxidant and antinociceptive effects on the various models tested. The antioxidant effect of the extract and pure compound was evaluated using different assays such as DPPH, deoxyribose damage and lipid peroxidation. The leaves extract (BCL) of *B. ceiba* showed promising antioxidant properties with DPPH assay. On its division into BCM and pure compound **1** (mangiferin), the activity appeared in **1**, while acetyl[13] (**1a**) and cinnamoyl (**3**) deriva-

tives of **1** possessed reduced activity. The methyl[14,15] (**2**) and its acetyl derivative[15] (**2a**) are found to be inactive (Table 1). Based on antioxidant IC_{50} values 5.56±0.33 μg/ml (rutin), 5.8±0.96 μg/ml (**1**), 13.5±1.78 μg/ml (**3**), 14.2±1 μg/ml (**1a**), 15.75±1.43 μg/ml (ascorbic acid), 20.12±2.16 μg/ml (BCL) and 52±2.45 μg/ml (BCM), the corresponding potency order appears to be rutin=**1**>**3**≥**1a**>ascorbic acid>BCL≫BCM. It is noteworthy that mangiferin and rutin (a standard antioxidant) demonstrated similar magnitude of activities as reflected by their IC_{50} values (5.80±0.96 μg/ml *versus* 5.56±0.33 μg/ml). Previously, in the DPPH assay mangiferin bearing a catechol moiety with a 6,7-dihydroxylated structure has been proposed to be responsible for antioxidant property[23] that was comparable to *dl*-α-tocopherol. Apparently, antioxidant property could be correlated to the number of hydroxyl groups and catechol moiety in the molecule, and mangiferin in our studies appears to be a better antioxidant compared to **1a** and **3** which bear no 6,7-dihydroxylated structure (catechol moiety). However, the presence of methoxy groups as in **2** and **2a** abolishes the antioxidant activity as both these compounds failed to demonstrate antioxidant property even at 200 μg/ml. This observation is in complete accordance with the conclusions drawn from a larger study on 40 flavones and flavonol.[24] Furthermore, mangiferin could not significantly protect against either deoxyribose damage or lipid peroxidation. In both these assays extracts and pure compound showed weak antioxidant activities (data not shown). A possible reason for these results is that mangiferin has ability to scavenge free radicals formed in the initial step of lipid peroxidation as has also been noticed previously in rat liver microsomes.[23] It is more likely that in the case of deoxyribose degradation system similar situation may also be operative. Additionally, in our assays, rutin also demonstrated negligible activity. All the antioxidant assays conducted demonstrate remarkable similarities between mangiferin (xanthone) and rutin (flavonoid). It is interesting to note that in the literature, on the basis of its distribution and biogenesis mangiferin is described more closely to the flavonoids than to other xanthone derivatives.[25,26] This may be explained on the basis of the facts that mangiferin occurs in some plants in the presence of C-glucosyl flavones rather than with other xanthones.[26—28] Moreover, xanthone nucleus of mangiferin is indeed formed from a flavonoid type C_6C_3 precursor (*p*-hydroxycinnamate) coupled with two malonates. The labeled benzophenones were significantly incorporated into mangiferin, whereas labeled 1,3,6,7-tetrahydroxy xanthone was not, suggesting that the glycosylation occurs at the benzophenone stage.[27,29] Benzoic acid is apparently not on the pathway as observed in case of biosynthesis of other simple xanthones,[29] therefore, the biosynthetic pathway leading to mangiferin is more related to that found for the flavones, than that of the normal xanthones.[27,29]

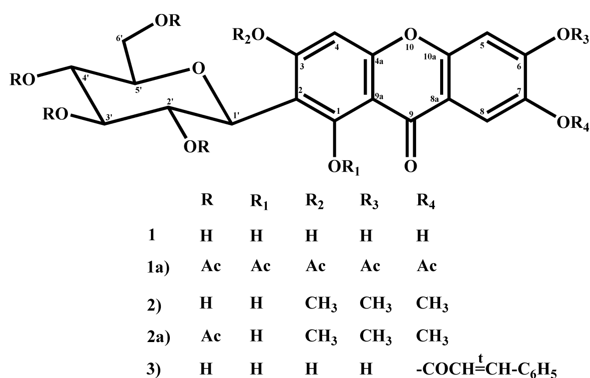

Fig. 1. Mangiferin and Its Derivatives

Keeping in mind the *in vitro* antioxidant properties of mangiferin it was extended for further confirmation under *in vivo* conditions. For this purpose mangiferin was subjected to CCl_4-induced hepatotoxicity. The estimated levels of serum ALT and AST in the control animals were found to be 34.6±3.9 IU/l and 154±15.5 IU/l, respectively, which were significantly raised to respective values of 152.84±25.4 IU/l and 319.72±88.23 IU/l after administration of CCl_4. This substantial rise is an indicative of cellular leakage and loss of functional integrity of cell membranes in liver.[19,20] It was accompanied by discolouration of liver that was evident upon autopsy of the animal. Pretreatment of animals with BCL and BCM (1—100 mg/kg) did not cause any change in the enzyme levels, whereas, after treatment with mangiferin (0.1, 1, 10 mg/kg) both enzymes showed corresponding decline of about 34%, 47% and 62% which are significantly lower than the CCl_4 treated animals (Fig. 2). In ethanolic extract of *Polygala elongata* Klein Ex. Willd, mangiferin was suggested to be responsible for hepatoprotective effect.[30] It appears that mangiferin due to its antioxidant nature may have trapped the damaging molecules making them unavailable to cause the cellular damage. Alternatively, inhibitors of drug metabolizing enzymes can also disrupt CCl_4 bioactivation into reactive species, thereby providing protection against hepatocellular damage. It is well established that strychnine is a substrate for microsomal drug metabolizing enzymes (MDME) and

Table 1. DPPH Scavenging Activity of *Bombax ceiba* Extract, Mangiferin (**1**), **1a**, **3**, Rutin and Ascorbic Acid

Dose (μg/ml)	BCL	BCM	Mangiferin (**1**)	BCP-AC (**1a**)	BCPC-1E (**3**)	Rutin	Ascorbic acid
2	ND	ND	23.92±2.15	ND	ND	22.66±4.7	ND
5	ND	ND	44.47±6.24	16.89±1.19	21.96±4.64	46.11±4.4	17.30±1.37
10	31.06±4.42	14.82±0.60	77.24±7.21	35.85±1.55	40.96±6.89	85.07±2.01	39.45±3.88
15	40.50±4.15	ND	83.75±3.6	61.16±3.73	ND	ND	48.50±1.24
20	49.84±4.44	22.48±1.24	92.79±0.54	80.61±1.51	71.37±9.58	86.00±1.21	57.62±1.90
30	68.35±5.25	35.19±1.95	93.56±0.05	92.43±0.17	78.36±11.5	ND	ND
50	93.09±1.07	51.73±5.41	ND	92.80±0.53	89.25±0.09	89.77±1.67	65.56±1.80
IC_{50}	20.12±2.16	52.00±2.45	5.80±0.96	14.20±1.00	13.50±1.78	5.56±0.33	15.75±1.43
			13.74 μM	18.73 μM		9.11 μM	79.54 μM

Potency order=Rutin=mangiferin (**1**)>**3**≥**1a**>ascorbic acid>BCL≫BCM. **1a**=Acetyl derivative of mangiferin. **3**=Cinnamoyl derivative of mangiferin. **2** methyl derivative of mangiferin and **2a** acetyl derivative of **2** displayed no activity till 200 μg/ml. ND=not done.

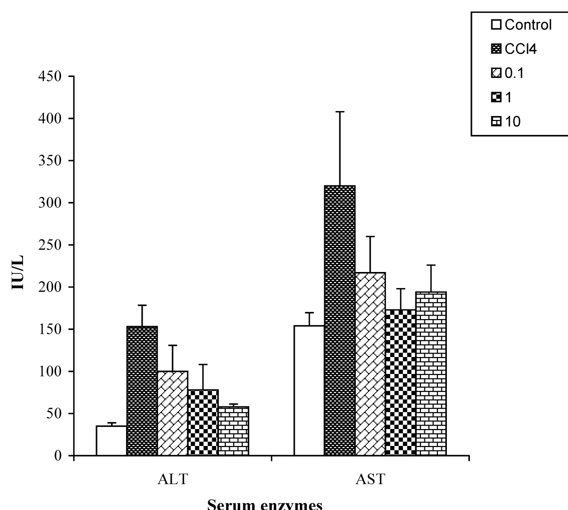

Fig. 2. Effect of Pretreatment with Mangiferin (0.1, 1, 10 mg/kg) on CCl_4-Induced Increase in Serum Transaminases (ALT and AST) Levels in Rats

Each bar represents mean±S.E.M. of 6—10 determinations. All the enzyme levels were significantly ($p<0.05$) different from the CCl_4 treated animals. Pretreatment with BCL and BCM (1—100 mg/kg) on CCl_4-induced effect did not cause any change in the enzyme levels (not shown graphically).

most known inhibitors of this enzyme increases the toxicity of strychnine through potentiation of its CNS stimulant activity.[20] The lack of mortality of mice at the sublethal dose of strychnine in the presence of mangiferin (data not shown) indicated that mangiferin is not an inhibitor of MDME. That, mangiferin also failed to inhibit NADPH-dependent cytochrome P-450 reductase activity.[23] This fact further strengthens the hypothesis that hepatoprotective activity is more likely to be mediated due to its inherent free radical scavenging nature.

In the present study analgesic activity of the leaves extract and mangiferin were also evaluated by the acetic acid induced writhing test and hot plate test. These tests allow to analyze peripheral and centrally mediated antinociceptive responses. In the writhing test, the methanolic extract of *B. ceiba* leaves (BCL) its fraction (BCM) and mangiferin (**1**) induced a significant and dose dependent reduction in the number of writhes in mice compared to control animals (Table 2a). At 100 mg/kg the extract caused about 70% reduction in the number of writhes, whereas, at the same dose aspirin demonstrated about 10% greater inhibition. The IC_{50} values indicate that upon division of BCL into BCM and **1**, analgesic activity was accumulated in (**1**) which appears to be slightly better than aspirin (18.2±2.0 mg/kg).

Table 2b depicts that the acetic acid induced pain was antagonized in the presence of morphine (65%) and mangiferin (70%), however, in the presence of naloxone, the corresponding effects were only 19% and 28%, suggesting that mangiferin, like morphine, is effective in abolishing acetic acid induced pain in an opioid way.

To determine the possible mechanism of analgesic action, hot plate test that has selectivity for opioid derived centrally mediated analgesia was used.[31] Animals treated with BCL, BCM or mangiferin, showed significantly longer latency than the control group after 90 min (Table 3). No significant latency was observed at time 0, 30 and 60 min with any dose of either extract or mangiferin, whereas morphine (10 mg/kg) used as reference drug, had an antinociceptive effect at all times tested (30, 60, 90, 120 min) compared to the control. For the sake of clarity the complete data is not represented in Table 3. At 120 min only the analgesic effect for BCL was significant. All these results indicate that BCL, BCM and mangiferin causes analgesia by their action at central nervous system but BCL-induced effect is comparatively long lasting.

Table 2a. Effect of *Bombax ceiba* Extracts, Mangiferin (**1**) and Aspirin on Acetic Acid-Induced Writhing Test in Mice

Treatment	Dose (mg/kg)	% Inhibition of writhes	IC_{50} (mg/kg)
BCL	10	31.62	41.33±4.6
	50	52.13	
	100	72.91	
BCM	10	27.44	64.00±6.0
	50	41.41	
	100	71.13	
Mangiferin (**1**)	0.42	38.89	12.53±3.0
	4.22	46.65	
	42.20	59.29	
Aspirin	10	37.10	18.20±2.0
	30	68.50	
	100	80.02	

Number of writhes in control animals (10 ml/kg, 0.9% acetic acid)=100.47±8.25. Number of mice per dose=10. Test substances were administered intraperitoneally before the administration of acetic acid. All the values were significantly ($p<0.05$) different from the control. Potency order=mangiferin (**1**)>aspirin>BCL>BCM.

Table 2b. Effect of Mangiferin (**1**) and Morphine on Acetic Acid-Induced Writhes in the Presence and Absence of Naloxone in Mice

Treatment	Dose (mg/kg)	% Inhibition of writhes
Without naloxone		
Mangiferin (**1**)	42.20	69.7
Morphine	0.25	65.4
With naloxone	5.00	
Mangiferin	42.20	28*
Morphine	0.25	18.57*

Number of writhes in control animals (10 ml/kg, 0.6% acetic acid)=46.25±2.68. Number of mice per dose=10. Test substances were administered sub-cutaneously before the administration of acetic acid. The asterisk represents significant difference ($p<0.05$).

Table 3. Effect of *Bombax ceiba* Extracts, Pure Compound Mangiferin (**1**) and Morphine in Hot Plate Test in the Presence and Absence of Naloxone

Test substance	Dose (mg/kg)	Response time (seconds)		
		0 min	90 min	120 min
Without naloxone				
Control (saline)		6.0±1.54	5.50±1.99	5.60±1.6
BCL	100	6.2±2.20	12.25±3.61*	11.62±2.26*
BCM	100	6.0±1.91	11.57±4.86*	10.71±3.30$^{n.s}$
Mangiferin (**1**)	42.2	6.6±2.08	14.0±4.89*	10.0±4.07$^{n.s}$
Morphine	10	6.0±1.85	15.25±3.88*	11.37±2.92*
With naloxone	2			
BCL	100	8.6±1.24	12.75±2.23$^{n.s}$	14.25±2.36$^{n.s}$
BCM	100	12.4±3.21	12.60±2.88$^{n.s}$	14.20±1.9$^{n.s}$
Mangiferin (**1**)	42.2	10.0±0.7	8.75±1.48$^{n.s}$	8.40±1.85$^{n.s}$
Morphine	10	5.6±2.07	6.0±2.34*	6.0±1.58$^{n.s}$

Without naloxone: All the values were compared with control (10% DMSO). With naloxone: It was injected 15 min prior to administration of test compound. All the results were compared with their respective test substances in the absence of naloxone. n.s.=non-significant and asterisk represents significant difference ($p<0.05$).

To further explore the mechanism of analgesic action, naloxone, a non-selective antagonist of opioid receptors was used. It is established that naloxone acts by antagonizing the action of endogenous opioids involve in pain or stress. The data showed that naloxone (2 mg/kg) reversed about 38% antinociceptive effect of the mangiferin (statistically non-significant) but the BCL and BCM induced effects remained unchanged in its presence. Morphine-induced analgesia, was completely reversed in its presence. These results suggest that BCM, BCL and mangiferin caused analgesic effect. However, BCL and BCM elicited effect is completely independent of opioid receptors but in case of mangiferin the involvement of opioid receptors cannot be ignored. Additionally, BCM with naloxone at 0 time showed significantly greater latency period as compared to its control. This unusual transient observation is difficult to explain.

In the present investigation BCL, BCM and mangiferin at 100 mg/kg failed to exhibit detectable anti-inflammatory activity when subjected to carrageenan-induced rat paw edema, a popular screen for the evaluation of anti-inflammatory properties[32)] implying that they are devoid of acute anti-inflammatory activity. Although, this result is negative but it is not contradictory to the acclaimed use of the plant against chronic inflammation. Unlike, non-immunological carrageenan assay used in the present study, marked reduction on the phagocytic production of rat macrophages by mangiferin from *Mangifera indica* L. extracts revealed that anti-inflammatory response is detectable but after the onset of the immunological response.[33)]

The extracts and pure compounds **1** and **1a** were also evaluated in National Cancer Institute (NCI, NIH, Bethesda, U.S.A.) for anticancer and anti-HIV activities.[34)] All the samples were found to be inactive as cytotoxic (against panel of 60 cancer cell lines) and as anti-HIV agent. On the contrary, inhibitory effects of mangiferin in azoxymethane-induced rat colon carcinogenesis indicated its chemoprotective nature.[35)] This discrepancy may have arisen due to the differences between the *in vitro vs. in vivo* assays used to assess the anticancer activity and thereby emphasizes the need to conduct a combination of both types of assays before reaching a definite conclusion. It is important to note that, swertipunicoside, a mangiferin derivative, showed the anti-HIV reverse transcriptase activity.[36)]

On the basis of the current investigation, we may state that BCL, BCM and mangiferin from *B. ceiba* possess antioxidant and analgesic properties. In certain cases the antinociceptive activity is probably *via* the participation of the opioid receptor interaction. Further investigations are necessary to elucidate the detailed mechanism of the analgesic effect residing in the extracts and mangiferin.

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