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Anti-inflammatory, anti-ulcer and hypoglycaemic activities of ethanolic and crude alkaloid extracts of *Madhuca indica* (Koenig) Gmelin seed cake

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SUMMARY

Madhuca indica has been used ethnomedically in Indian folks. In the present study we have investigated anti-inflammatory, anti-ulcer and hypoglycaemic effect of ethanolic extract (EE) and crude alkaloid extract of *Madhuca indica* seed cake on albino rats. The study showed that the EE had a significant, dose dependent anti-edematogenic, anti-ulcerogenic and hypoglycaemic activity, whereas the crude alkaloid extract exhibited a significant anti-inflammatory activity only. Both the extracts possess dose dependent inhibitory activity on carrageenan-induced edema, inhibiting prostaglandins or mediators involved in prostaglandin synthesis, the second phase of inflammation. The EE was significantly effective in protecting pylorus-ligation-induced gastric ulcers at a higher dose level. The active principle of EE seems to be a selective inhibitor of the COX II (prostaglandin synthesis) without important effect on COX I since, EE exhibited both anti-edematogenic and anti-ulcerogenic effect. The EE was effective in reducing the plasma glucose level in normal albino rats in a dose dependent manner, producing hypoglycaemic effect by stimulating the release of insulin from the β-cells and/or increasing the uptake of glucose from the plasma.

Key words: *Madhuca indica* seed cake; Anti-inflammatory activity; Anti-ulcer activity; Hypoglycaemic effect; Ethanolic extract; Crude alkaloid extract

INTRODUCTION

Madhuca indica (Koenig) Gmelin syn. *Madhuca longifolia*, *Madhuca latifolia, Bassia latifolia* belongs to the family Sapotaceae, commonly known as mahua. Mahua is a large, shady, deciduous tree doting much of the central Indian landscape, both wild and cultivated. The tree is valued for its oil-bearing seeds and flowers, which are utilized for alcoholic beverage production. Mahua seeds are of economic importance as they are good source of edible fats (Ramadan *et al*., 2005). The expectorant flowers are used to treat chest problems such as bronchitis and also taken to increase of breast milk. The distilled juice of the flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, pharyngitis (Nadkarni, 1954) as well as bronchitis (Varier, 1995). The leaves are applied as a poultice to relieve eczema. In Indian folk medicine, the leaf ash is mixed with ghee to make a dressing for wounds and burns. Mahua preparations are used for removing intestinal worms, in respiratory infection and in cases of debility and emaciation. The astringent bark extract is used for dental-related problems, rheumatism and diabetes (www.herbnet.

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com). The fat obtained from mahua seeds has many medicinal applications. The seed fat has emuluscent property, used in skin disease, rheumatism, headache, laxative, piles and sometimes as galactogogue. The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent. The bark is a good remedy for itch, swelling, fractures and snake-bite poisoning, internally employed in diabetes mellitus, fruits are astringent and largely employed as a lotion in chronic ulcer, in acute and chronic tonsillitis and pharyngitis.

Previous phytochemical studies on *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides (Yosiokal *et al*., 1974; Yoshikawa *et al*., 2000). In view of the attributed medicinal properties, new components including: madhucic acid (a pentacyclic triterpenoid), madhushazone (a untypical isoflavone), madhusalmone [a bis(isoflavone)] (Yoshikawa *et al*., 2000), four new oleanane-type triterpene glycosides (madlongisides A-D) (Siddiqui *et al*., 2004) and madhucosides A and B were recently isolated and characterized from *Madhuca indica*. Madhucosides A and B showed significant inhibitory effects on both superoxide release from polymorphonuclear cells in a NBT reduction assay and hypochlorous acid generation from neutrophils assessed in a luminol-enhanced chemiluminescence assay (Pawar and Bhutani, 2004).

Mahua seed cake (defatted) is however poisonous and as such it has only limited use as insecticide, piscicide or manure. In view of this collection and utilization of seed is low (Mulky and Gandhi, 1977). So an attempt was made to investigate the pharmacological activities of *Madhuca indica* seed cake extracts. To the best of our knowledge this is the first report on alkaloids from *Madhuca indica* seeds.

MATERIALS AND METHODS

Madhuca indica (Koenig) Gmelin seeds were collected from konchavaram forest, Gulbarga district, Karnataka, India, during June-July 2004 and authenticated by Dr. Y.N. Seetharam, faculty of Botany, Gulbarga University, Gulbarga and a voucher specimen was deposited in the herbarium (HGUG No. 723). The seeds were air dried and powdered.

Extraction

Known weight of powdered seed material was defatted with petroleum ether (40 - 60°C) and the defatted seed material were divided into two equal fractions. Fraction 1 was subjected to soxhlet extraction by 95% ethanol for 36 h and the extract was evaporated under controlled temperature (40 - 50°C) to a small volume. The extract thus obtained was taken as ethanolic extract (EE); and was phytochemically screened (Trease and Evans, 1983). Fraction 2 was wetted with 500 ml of 50% ammonium hydroxide and lixiviated overnight with 5 ml of ethyl acetate. The lixiviate was extracted with 2% sulphuric acid and the aqueous phase made alkaline with ammonium hydroxide and extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulphate and evaporated in vaccuo to give crude alkaloid extract (CAE) (Thepenier *et al*., 1990). 1% gum acacia was used as vehicle.

Animals

Albino rats of either sexes (150 - 250 g) were obtained from National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore and were kept in standard plastic cages in a group of 6 - 8 in each cage at standard condition at 12 h of light and dark cycles and were fed with standard rodent diet and water *ad libitum*. After one week of acclimatization the animals were used for further experiments. Approval from the institutional animal ethical committee for usage of animals in the experiments was obtained.

Acute toxicity study

In vivo toxicity (Trease and Evans, 1983). The rats were randomly divided into four groups (n = 6).

Group 1 was administered with vehicle and group 2, 3 and 4 were given a dose level of 100, 75 and 50 mg/kg of both the extracts macerated with vehicle, respectively, via intragastric intubation. Animals were observed and the mortality rates were recorded within the first 24 h of dose administration. $1/10^{th}$ and $1/5^{th}$ of LD_{50} was considered for the studies.

Anti-inflammatory study

Carrageenan induced edema (Winter *et al*., 1962). The animals were divided into six groups (n = 5). In all groups, acute inflammation was produced by sub-plantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan in normal saline in right hind paw of the rats and the paw volume was measured plythesmometrically at 1, 2, 4 and 6 h after carrageenan injection. Animals were pre-dosed with 5 and 10 mg/kg of both the extracts. 0.2 ml vehicle and 40 mg/kg indomethacin were administered intraperitoneally (i.p) as negative and positive control, respectively, 30 min before the carrageenan injection. Mean increase in the paw volume was measured and the percentage inhibition was calculated with reference to negative control.

$$\text{Percentage inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Anti-ulcer study

Pylorus ligature (Shay *et al*., 1945). The animals were randomized into six groups (n = 5). Group 1 and 2 were administered with vehicle and 40 mg/kg lansoprazole, serving as negative and positive controls, respectively. Group 3, 4, 5 and 6 received 5 and 10 mg/kg of both extracts macerated with vehicle. After 30 min of dose administration, the abdomen was incised and the pylorus ligated under light ether anesthesia. 4 h after pylorus ligation, the animals were sacrificed and the stomach was removed. The gastric content was collected and centrifuged. The volume, pH, total and free acidity of gastric fluid determined. The stomach was then incised along the greater curvature and observed for ulcers. The coloration of the stomach was observed and the number of ulcers counted using a magnifying glass (10×). Mean ulcer score for each animal expressed as ulcer index. The ulcers were graded using the following scoring system:

0, normal mucosa
0.5, blushing
1, spot ulcers
1.5, hemorrhage streaks
2, ulcers ≥ 3 but ≤ 5
3, ulcers > 5

Hypoglycaemic activity

Hypoglycaemic activity of both the extract was evaluated (data presented and discussed is of ethanolic extract (EE) since, crude alkaloid extract (CAE) exhibited almost similar effect as that of vehicle). Over night fasted animals were divided into four groups (n = 5). Group 1 and 2 received vehicle and 40 mg of tolbutamide, serving as negative and positive control, respectively. Group 3 and 4 were orally administered 5 and 10 mg/kg of EE macerated with vehicle, respectively. Plasma glucose was estimated (GOD-POD method) using a commercial kit (Abot gluco-meter, USA.) at 0, 1, 2 and 4 hr and percentage variation of glycaemia was calculated for each group using the following formula:

$$\% \text{ variation of glycaemia} = \frac{G_1 - G_t}{G_i} \times 100$$

Where G_i and G_t were the values of initial glycaemia (0 h) and glycaemia at 1, 2 and 4 h respectively.

Statistical analysis

The results are presented as the mean ± standard error of the mean (S.E.M.), and were compared using one way analysis of variance, followed by Dunnet's pairwise test. *P* values less than 0.05 were considered significant.

RESULTS

The phytochemical screening of the EE indicated the presence of alkaloids, saponins, flavonoids, tannins, phenols and glycosides. LD_{50} was found to be 50 mg/kg for both the extracts. The anti-edematogenic response obtained by the administration of the extracts, standard and vehicle on carrageenan-induced hind paw edema in rats is shown in Table 1. Both the extracts showed a significant reduction in the paw edema, however the anti-edematogenic response was dose dependent. The inhibitory response of both the extracts was more significant from the second hr and reached maximum in the fourth, and then decreased gradually. Although both the doses of CAE showed significant increase in % reduction of paw volume when compared to vehicle, 10 mg/kg dose was more effective in inhibiting the edema, particularly from the second h (64.70) and equaled with that of indomethacin in the fourth h (60.31). The anti-edematogenic response exhibited by EE (5 mg/kg) was similar with that of indomethacin, particularly at fourth (60.31) and sixth (55.17) h. EE (10 mg/kg) showed a far better significant response compared to indomethacin, starting from the second h (67.64) and exceeded indomethacin in the fourth (68.25) and sixth (67.24) h. Indomethacin significantly inhibited edema from the first h (42.10) after carrageenan injection, but the response was not prolonged especially after second h where the inhibition was maximum (72.05), and then decreased progressively, whereas the EE (10 mg/kg) showed significant inhibitory response from the second h (67.64) and the anti-edematogenic effect was almost consistant till sixth h.

Table 2. Effect of ethanolic and crude alkaloid extracts of *Madhuca indica* seed cake on pylorus-ligation-induced gastric ulcer in rats

Treatment	Dose (mg/kg)	Ulcer index
Vehicle	-	4.20 ± 0.48
Lansoprazole	40	0.90 ± 0.24*
CAE	5	3.80 ± 0.51
CAE	10	2.20 ± 0.78
EE	5	2.80 ± 0.46
EE	10	1.20 ± 0.30*

Values are mean ± S.E.M.; *$P < 0.05$.

The anti-ulcerogenic effect of the extracts on pylorus-ligation-induced gastric ulcer is presented in Table 2. The EE at a dose level of 10 mg/kg showed a significant decrease in the ulcer index (1.20) compared to vehicle, and was near to that of lansoprazole used at a dose level of 40 mg/kg, while all other extracts of *Madhuca indica* exhibited no significant gastro-protective effects.

The hypoglycaemic effect of the EE was highly dose dependent Table 3. The hypoglycaemic effect of both the extracts were observed as early as 1 h (13.99, 50.00) after administration at all the doses compared to vehicle. The hypoglycaemic effect of EE (10 mg/kg) was much more pronounced than tolbutamide. At 5 mg/kg dose, the extract showed

Table 1. Effect of ethanolic and crude alkaloid extracts of *Madhuca indica* seed cake on carrageenan-induced hind paw edema in rats

Treatment	Dose (mg/kg)	Mean paw – Size in mm				% Inhibition			
		1 h	2 h	4 h	6 h	1 h	2 h	4 h	6 h
Vehicle	-	0.38 ± 0.003	0.68 ± 0.004	0.63 ± 0.008	0.58 ± 0.007	-	-	-	-
Indomethacin	40	0.22 ± 0.014*	0.19 ± 0.007*	0.25 ± 0.008*	0.26 ± 0.003*	42.10	72.05	60.31	55.17
CAE	5	0.32 ± 0.008*	0.38 ± 0.020*	0.31 ± 0.007*	0.51 ± 0.011*	15.78	44.11	50.79	12.06
CAE	10	0.27 ± 0.013*	0.24 ± 0.015*	0.25 ± 0.015*	0.32 ± 0.003*	28.94	64.70	60.31	44.82
EE	5	0.25 ± 0.007*	0.36 ± 0.013*	0.25 ± 0.018*	0.26 ± 0.010*	34.21	47.05	60.31	55.17
EE	10	0.31 ± 0.003*	0.22 ± 0.007*	0.20 ± 0.004*	0.19 ± 0.009*	18.42	67.64	68.25	67.24

Values are mean ± S.E.M.; *$P < 0.05$.

Table 3. Effect of ethanolic extracts of *Madhuca indica* seed cake on blood glucose levels of normal rats

Treatment	Dose (mg/kg)	Blood glucose (mg/dl)				% Glycaemia			
		0 h	1 h	2 h	4 h	0h	1 h	2 h	4 h
Vehicle	-	80.40 ± 0.19	83.46 ± 0.56	79.67 ± 0.65	81.65 ± 0.71	-	-	-	-
Tolbutamide	40	92.30 ± 0.97	61.00 ± 0.69*	49.80 ± 0.68*	43.30 ± 0.70*	-	33.91	46.04	53.08
EE	5	81.41 ± 1.31	70.02 ± 1.30*	46.52 ± 0.57*	45.50 ± 0.31*	-	13.99	42.85	44.11
EE	10	88.00 ± 1.19	44.00 ± 0.70*	33.20 ± 0.48*	22.04 ± 0.67*	-	50.00	62.27	74.95

Values are mean ± SEM; *$P < 0.05$.

a significant hypoglycaemic effect, however the reduction in plasma glucose level was not as pronounced, compared to tolbutamide and 10 mg/kg EE dose. EE at 10 mg/kg dose level had a significant hypoglycemic effect. The hypoglycaemic effect shown by EE (10 mg/kg) at the first h (50.00) was almost comparable to that shown by tolbutamide (40 mg/kg) at fourth h (53.08), which kept on increasing till the fourth h (74.95).

DISCUSSION

The acute toxicity studies indicated that the LD_{50} of EE and CAE was 50 mg/kg when administered orally. The LD_{50} for alcoholic extract of *Madhuca indica* flowers was 160 mg/kg (Dinesh, 2001). Whereas the LD_{50} of purified seed saponin were 1 g/kg, 15 mg/kg and 15 - 20 mg/kg, for oral, intravenous and intraperitoneal administration, respectively. The mechanism of toxicity by parenteral route can be explained on the basis of massive haemolysis produced by the saponin causing death due to anoxia. When administered orally the saponin is perhaps not absorbed directly but causes destruction and sloughing of the superficial layers of the intestinal mucous membrane followed by intense inflammation and some degree of absorption into circulation through damaged hyperaemic tissues. (Mulky and Gandhi, 1977).

The most used test to study new anti-inflammatory agents evaluates the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent (Winter *et al*., 1962). Subcutaneous injection of carrageenan into the rat paw produces plasma extravasation (Szolcsanyi *et al*., 1998) and inflammation is characterized by increased tissue water and plasma protein exudation with neutrophil extravasation and metabolism of arachidonic acid by both cyclo-oxygenase and lipoxygenase enzyme pathways (Gamache *et al*., 1986). There are biphasic effects in carragenan-induced edema. The first phase begins immediately after injection and diminishes within 1 h. The second phase begins at 1 h and remains through 3 h (Garcia-Pastor *et al*., 1999). It is suggested that the early hyperemia of carrageenan-induced edema results from the release of histamine and serotonin (Kulkarni *et al*., 1986). Prostaglandins play a major role in the development of the second phase of reaction (Crunkhon and Meacock, 1971; DiRosa and Willoughby, 1971; DiRosa, 1972). In the present investigation both the EE and CAE of *Madhuca indica* showed a significant and dose dependent anti-inflammatory activity. Lower doses of both the extracts also showed anti-inflammatory effect, but higher doses were far more effective in inhibiting the edema, especially the EE 10 mg/kg showed anti-edematogenic response better than the reference drug indomethacin. Both the extracts showed significant anti-inflammatory activity from the second hr of extract administration indicating that the active principle in both the extract is inhibiting mainly the second phase of carrageenan induced edema, either inhibiting prostaglandins synthesis or preventing the release of inflammatory mediators involved in the synthesis of prostaglandins. Indomethacin

seems to act by inhibiting both the phases of carrageenan induced edema however, regarding the doses of 40 mg/kg for indomethacin, EE 10 mg/kg was a more potent anti-inflammatory compound than indomethacin for the second phase of inflammation.

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrythmias, impotence, gynaecomastia and haematopoeitic changes) of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer (Akhtar *et al*., 1992). Currently, proton pump inhibitors such as omeprazole are extensively used to control increased acid secretion and acid related disorders including gastroesophageal reflux disease, Zollinger-Ellison syndrome and gastroduodenal ulcers caused by stress (stress related erosive syndrome), nonsteroidal anti-inflammatory drugs and by *H. pylori* (Langtry and Wilde, 1998; Horn, 2000; Wolfe and Sachs, 2000). Although histamine-H_2 receptors blockers (ranitidine, famotidine etc.) and proton pump inhibitors (omeprazole, lansoprazole etc.) have been used for the efficient management of gastric hyperscretion and gastroduodenal ulcers, several adverse effects of these drugs have also been reported (Havu *et al*., 1990; Wandall, 1992; Kakei *et al*., 1993; Howden and Hunt, 1994; Peterson, 1994; Fort *et al*., 1995; Powers *et al*., 1995; Shindkawa *et al*., 1996; Martelli *et al*., 1998). Drugs with multiple mechanism of protective action, including anti-oxidant properties, may be one way forward in minimizing tissue injury in human diseases (Barry, 1991). Although in most of the cases the aetiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defence mechanism (Piper and Stiel, 1986). Studies have shown alteration in the anti-oxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus-ligation induced ulceration in rats (Rastogi *et al*., 1998). In the present investigation only the EE 10 mg/kg exhibited a significant gastro protective effect. It has been postulated that histamine might be involved in the formation of pylorus-ligated ulcers and plays a mediating role in the gastric secretion stimulated by gastrin, vagal excitation and cholinergic agents (Glick *et al*., 1966; Grossman and Konturek, 1974; Rangachari, 1975). Thus the effect of the EE on gastric lesions induced by pylorus ligation could be due to the histamine inhibition and or scavenging the free radical. This gastro-protective effect of the extract can be attributed to the various bioactive principle detected in the ethanolic extract. Pylorus ligation-induced ulcers are due to autodigestion of the gastric mucosa and break down of the gastric mucosal barrier (Sairam *et al*., 2002). Thus the active principle in the extract might be enhancing the mucosal defensive factors leading to increased mucus production protecting the surface epithelial cells. One of the draw back of NSAID's is mucosal damage by interfering with prostaglandin synthesis, increased acid secretion and back diffusion of H^+ ions (Rao *et al*., 2000), and the search for safe anti-inflammatory drug that is free from gastric intolerance continues unabated and a part of such research is the evaluation of medicinal plants known to be used for the treatment of inflammatory disorders (Singh *et al*., 1989). Since in our study the EE (10 mg/kg) showed a significant anti-inflammatory and anti-ulcer property, the active principle of the extract might be inhibiting prostaglandins by selective action on COX II, without an important effect on COX I (Antônio and Souza Brito, 1998).

More than 100 medicinal plants are mentioned in the Indian system of medicine including folk medicines for the management of diabetes, which are effective either singly or in combination. As pointed out by WHO, prevention of diabetes and its complication is not only a major challenge for the future, but essential if health for all is to be an attainable target. This WHO study groups emphasizes strongly in this regard the optimal, rational uses of

traditional and natural indigenous medicines (WHO, 1985; 1994). Surveys conducted in Australia and United States indicated that, respectively, 48.5 and 34% of the respondents used at least one form of unconventional therapy, including herbal medicine (Eisenberg *et al*., 1993; Maclennan *et al*., 1996). Tolbutamide acts by stimulating insulin secretion (pancreatic) (Vigneri *et al*., 1982) and also by increasing tissue uptake of glucose (extra pancreatic) (Peifer *et al*., 1981). It is evident from the results that the EE has a significant dose dependent hypoglycaemic activity in normal rats. The results suggest that the EE exhibited action similar to tolbutamide (reference drug) i.e. stimulating the release of insulin from â-cells and by increasing the uptake of glucose from the blood. This may be due to presence of various constituents like saponins, flavonoids and tannins. In many studies the hypoglycemic effect of these agents is already well documented. They have been reported to potentiate plasma insulin effect by increasing the pancreatic secretion of insulin from â-cells (Achrekar *et al*., 1991; Yanardag and Colak, 1998 ; Pari *et al*., 2001) and/or may increase the peripheral uptake of glucose (Bajaj and Srinivasan, 1999). It is a well-documented fact that most medicinal plants are enriched with bioflavonoids, which have antioxidant activity. Flavonoids as antioxidants exhibited several biological effects such as anti-hepatotoxic, anti-inflammatory, anti-allergic, anti-diabetic and anti-pyretic actions (Rajanarayana *et al*., 2001). The hypoglycaemic effect exhibited by EE 10 mg/kg was more significant than the reference drug tolbutamide. The percentage reduction in blood glucose level brought about by EE 10 mg/kg in the first hour was almost comparable to that shown by tolbutamide at a dose level of 40 mg/kg in the fourth h.

It can be concluded that *Madhuca indica* seed extracts possess anti-inflammatory, anti-ulcer and hypoglycaemic activities justifying its ethnomedical claims. Further investigations on isolating the active compound and the mechanism of action will be carried out, as *Madhuca indica* seems to be a promising candidate in the search for a safer drug considering its unique effect as an anti-inflammatory, anti-ulcerogenic and blood sugar levels reducing agent.

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