Full Length Research Paper

Antidiabetic activity of 7-methoxycoumarin from the bark of Marine plant *Rhizophora mucronata*

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Coumarin possess a number of biological activity like anticoagulant, antimicrobial, anti-inflammatory, analgesic, antioxidant, anticancer, antiviral, antimalarial etc. But the antidiabetic property of coumarins from marine plants are not attempted so far and hence, the present study has been carried out. The present study isolated a coumarins from the bark of *Rhizophora mucronata*. This coumarins belongs to a group as benzopyrones, which consists of a benzene ring joined to a pyronenucleus and confirmed by IR, ¹H-NMR, ¹³C-NMR and mass spectral studies. The present work deals with the isolation, characterization and antidiabetic activity of *in vivo* study of this compound. The isolated compounds have been identified as 7-methoxy coumarins and further, the isolated coumarins were subjected for the *in vivo* antidiabetic activity and it reveals that, the 7-methoxy coumarins potentiate antidiabetic activity.

**Keywords:** 7-methoxycoumarin, marine plant, antidiabetic property, *Rhizophora mucronata*.

INTRODUCTION

It has been estimated that diabetes may affect 439 million adults by 2030, with the major increase occurring in developing countries (Shaw *et al.*, 2010). It is projected that, it will rank as the 9th leading cause of death in low-income countries (Mathers and Loncar, 2006), Venables and Jeukendrup (2009)). There are two major types of diabetes, i.e. type 1 (T1D) or insulin dependent diabetes and type2 (T2D) cormman-insulin in dependent diabetes. The incidence of T2D is reaching epidemic proportions and has been in associated with an increase in obesity (Astrup, 2001; Li, 2003). According to the World Health Organisation (WHO) (Modak *et al.*, 2007) the main complications associated with diabetes are cardiovascular disease and renal failure.

*Rhizophora mucronata* (Rhizophoraceae) is a mangrove tree. The bark of this plant has been used by the local Thai people in a folk medicine for treatment of nausea, vomiting, diarrhea and stop bleeding in fresh wounds (Boonyapraphat and Chockchaicharaenphorn, 1998). This plant has been proved to possesses diterpenoids (Anjaneyulu *et al.*, 2002), triterpenoids (Ahmed *et al.*, 1998), andsteroids (Ghosh *et al.*, 1985), including alkaloids, phenolic compounds, coumarins, flavonoids, proteins and sugars, were analyzed by standard protocols (Siddiqui and Ali, 1997).

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Coumarin, a phenolic compound present in human dietary fruits and vegetables, is known to have antioxidant potential like vitamin E (α-tocopherol) and vitamin C for antioxidant have lipid lowering potential (Ramesh and Pugalendi, 2005). Derivative of coumarin, is a benzopyrone in nature and is present in the edible fruits, golden apple (Aeglemarmelos Correa) (Siddiqui and Ali, 1997), and bitter orange (Citrus aurantium) (Amrani et al., 2009). The parent compound coumarin has been reported to reduce blood glucose level (Yen et al., 2003). Cetto et al. (2000) have reported that UMB has antioxidant activity (Cetto and Wiedenfeld, 2001), but no detailed study has been carried out on the effect of UMB on blood glucose in streptozotocin (STZ)-diabetic rats. Hence, the present study was designed to investigate the effect of 7-methoxycoumarin on blood glucose of STZ-diabetic rats.

MATERIALS AND METHOD

Fresh sample bark of the R. mucronata were collected from the Karangkadu mangrove forest (Latitude 9°38' N and Longitude 78°57'E) in the Ramanathapuram district on the South East coast of India. Authentication of the plant species was done by Professor K. Kathiresan, Centre of Advanced Study in Marine Biology, Annamalai University, Porto Novo, Tamil Nadu, India. Voucher specimens of the sample has been maintained in the herbarium cabinet facility sponsored by the ICMR, New Delhi. Samples were washed three times with tap water and twice with distilled water to remove adhering salts and associated contaminants.

Dried barks of Rhizophora mucronata is pulverized and soaked in ethonal and water mixture (3:1) for one week and filtered by using muslin cloth. Further the extracts are concentrated under vacuum on a rotary evaporator, freeze-dried for solvent free residue and stored at -20°C until used. Isolation and identification of compound were carried out by the present study by following standard procedure. Increasing polarity 100% n-hexane, n-hexane-EtoAc, 100% EtoAc and 100% EtoH, to yield a number of fraction (fr) and further thin layer chromatographic (TLC) analyses were carried out. Fractions with Rf=0.61 brought maximum concentrations of extracts were subjected for spectral identification viz., NMR, LC-MS.

In vivo of hypoglycemic activity of 7-methoxy coumarins was also carried out by the present study. Male wistar rats and male swiss albino mice were maintained in standard hygienic laboratory conditions temperature around 26°C, day night cycle consisting of 12 and 12 hours. They were provided water and standard pellet food and water adlibitum. Animals were kept in standard polyprolene cages with hygienic conditions maintained. They were used after having obtained clearance from institutional animal ethics committee in Madurai Kamaraj University, Madurai (Reg no: IRBandEC/04.05.2012).

Induction of diabetes was done in overnight fasted male wistar rats by single intraperitoneal injection of streptozotocin, 50 mg.kg⁻¹ dissolved in 0.1M cold citrate buffer pH 4.5[19].48 hours after it blood samples were drawn by retro orbital route and blood glucose levels were checked to confirm diabetes (Cetto A, et al., 2000). Experiments were carried out with the following treatments (T1-T5) five animals were chosen for each treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
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<tbody>
<tr>
<td>T1-</td>
<td>Group 1- Saline alone (Negative control)</td>
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<tr>
<td>T2 -</td>
<td>Group 2 –Glibenclamide alone (10mg.kg⁻¹ p.o)</td>
</tr>
<tr>
<td>T3-</td>
<td>Group3 - 7-methoxycoumarine (500mg.kg⁻¹)</td>
</tr>
<tr>
<td>T4-</td>
<td>Group4- 7-methoxycoumarine (1000mg.kg⁻¹)</td>
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</tbody>
</table>

Blood samples were withdrawn from retro orbital route at 0, 60,120,180 mins [21] after the drug administration. Measurement of blood samples were done by using blood glucose kit.

RESULTS AND DISCUSSIONS

The infrared spectrum of 7-methoxy coumarin exhibits two strong bands at 1667 and 1340 cm⁻¹, which may be assigned to \( \nu(C=O) \) and \( \nu(C=O) \), respectively. The low carbonyl frequency (1667 cm⁻¹) is presumably due to intermolecular hydrogen bonding of the 7-hydroxyl hydrogen either with O (1) or with O (2). The vas(C O) (phenolic) has been assigned at 1561 cm⁻¹ (Boonyapraphat and Chokchaichaaraenphorn, 1998) as a very strong band. In the IR spectra of diorganotin (IV) derivatives of umbelliferone, there is a significant shift in vas(C O) (phenolic) (1604– 1610 cm⁻¹), which indicates the participation of the phenolic oxygen after the deprotonation of phenolic group in bonding with organotin moiety (Figure1) below.
In the $1H$ NMR (300MHz DMSO-d6) spectrum of the compound, six distinct peaks were observed. A broad singlet at $\delta 4.53$ was assigned to the OH proton. Two doublets at $\delta 6.12$ and 7.79 with coupling constant of 9.3 Hz were assigned to the protons attached at the C-3 and C-4 positions. Similarly, two other doublets, at $\delta 6.73$ ($J = 8.4$ Hz), $\delta 7.39$ ($J = 8.7$ Hz) and a singlet at $\delta 6.64$, were assigned to the protons attached to C-6, C-5 and C-8, respectively (Figure 2).
The $^{13}$C NMR spectrum of AN-2 showed nine distinct signals. A peak at δ 162.3 was assigned to the carbonyl function (C-2) of coumarin derivatives while a downfield signal at δ 161.7 revealed the presence of a methoxy function at the C-7 position. Other signals at δ 102.0, 110.9, 111.7, 113.1, 129.2, 144.6 and 155.8 were attributed to C-8, C-3, C-4a, C-6, C-5, C-4 and C-8a, respectively (Figure 3).

The mass spectra analysis 7-methoxycoumarin, the peak at m/z = 177.08[M+1] amu Na metal adopt in corresponds to the molecular ion peak (Figure 4).
Decreased Glucose level in T2 treated with 7-methoxy coumarine at 500 mg.kg$^{-1}$ concentration.

![Graph showing glucose levels in T1, T2, and T4 over time.](image1)

**Figure 5**

Decreased Glucose level in T3 treated with 7-methoxy coumarine at 1000 mg.kg$^{-1}$ concentration.

![Graph showing glucose levels in T1, T3, and T4 over time.](image2)

**Figure 6**
CONCLUSION

Structure prided of (fr-4) compound is 7-methoxy coumarin and anti-diabetic activity studies of both in vitro and in vivo is more activity. In vitro antidiabetic activity was found maximum in the basic medium pH added with coumarin when compared with the other two pH (Figures 5-7).

In in vivo animal modal, changes in blood glucose level was found normal, diabetic mice with 7-methoxycoumarin compound, Glibenclamide are presented in Figure 8. The results of the antidiabetic analysis suggested that, the glucose level decreases (187.6±1.304 in 180 min) in group 3 treated animals, which is highly comparable to positive control Glibenclamide (157.3±6.39 in 180 min).

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