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Antidiabetic and Antihyperlipidemic Effects of *Rhizophora apiculata* Blume Extracts

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Abstract

Objective: The present study aimed to evaluate the antidiabetic and antihyperlipidemic activities of ethanolic leaves extracts of *Rhizophora apiculata* and its dichloromethane and aqueous basic fraction in experimental diabetic rats.

Methods: The leaf materials of *R. apiculata* extracted with 80% ethanol and determined their phytochemicals constituents. Insulin-dependent diabetes mellitus (IDDM) was induced by single intraperitoneal injection of streptozotocin (60 mg/kg body weight), and Non-IDDM was induced by single intraperitoneal injection of streptozotocin (60 mg/kg body weight), after 15 min 120 mg/kg of nicotinamide injected intraperitoneally. The rats administered with oral doses of 250 mg/kg body weight of the ethanolic extract, dichloromethane (DCM-F) and aqueous basic fraction (AB-F) of *R. apiculata* up to 21 days respectively. The blood glucose level estimated on 0th, 7th, 14th, and 21st day by one touch glucometer. On the 21st day, the level of serum cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were evaluated.

Results: The phytochemical studies specified the alkaloids, flavonoids, and terpenoids present in the extracts. The rats treated with dichloromethane fraction indicated significant reduction in blood glucose ($P < 0.01$), serum cholesterol ($P < 0.05$) and triglycerides ($P < 0.05$) levels whereas HDL-C level was found to be increased ($P < 0.05$) as compared with the diabetic control group in both types.

Conclusion: Dichloromethane fraction of *R. apiculata* has prominent antidiabetic effect in IDDM and NIDDM experimental models. Further studies will confirm the active constituent in this fraction.

Keywords: dichloromethane, diabetes mellitus, high density lipoprotein, insulin, mangrove

1. Introduction

Diabetes mellitus and lipid abnormalities are one of the leading health issues in India and developing countries [1]. Currently available therapeutic options for diabetes mellitus, such as dietary modification, oral hypoglycemic agents, and insulin, have limitations of their own. There are two kinds of insulin sensitizer used clinically Thiazolidinediones and biguanides. They both play a crucial role in the treatment of NIDDM. However, with the widespread use of these agents, new problems have gradually arisen. Thiazolidinediones had many antagonistic effects, including the risk of cardiovascular adverse events, bone fractures, bladder cancer, and hepatotoxicity. For this reason, biguanides have become the choice for insulin sensitizer. The major side effects of metformin, biguanides, are gastrointestinal symptoms and a metallic taste in the mouth [2]. As a result, in actual clinical practice, if a patient is intolerant of metformin, there are no other insulin sensitizers available as an alternative.

An impressive number of modern drugs derived from plants, many of this isolate were based on the uses of the agents in traditional medicine [3]. There are a number of plant extracts, including mangroves and associated plants proved their hypoglycemic effect on animal models with fewer side effects [4, 5]. Therefore, the present study, we have chosen one of the traditionally valuable mangrove plants to evaluate their antidiabetic effect. *R. apiculata* Blume (Family: Rhizophoraceae) is used to treat astringent, diarrhea, pain and inflammation in Southeast coast of India [6]. Studies reported that *R. apiculata* extracts had anti-cholinesterase, anti-plasmodial, anti-viral antioxidant, anti-microbial, anti-diabetic, anti-nociceptive, α -glucosidase, and free radical scavenging activities [7-16]. Metabolites such as benzoquinone, campesterol, cinnamate, and lupeol, essential oils, sitosterol and stigmasterol reported in *R. apiculata* [17]. The oral medications were intended to have a systemic effect, reaching different parts of the body via the bloodstream. Earliest reports suggest that the oral administration of the plant extracts reduced blood glucose concentrations in experimental animals, possibly by interfering with food consumption and gastrointestinal absorption of food. Based on the earlier

scientific reports, the present work investigated the antidiabetic and antihyperlipidemic activities of ethanolic extract of leaves of *R. apiculata* and its dichloromethane and aqueous basic fraction in diabetic animal models.

2. Materials and Methods

2.1. Chemicals

Streptozotocin (STZ) and nicotinamide were purchased from Sigma, St. Louis, USA. Chemicals and solvents used were of analytical grade (Hi-media, Mumbai, India). The diagnostic kits for triglycerides, total cholesterol, and HDL-C were obtained from Asritha Diotech, Hyderabad, India.

2.2. Plant Material

Fresh leaves of *R. apiculata* were collected from Kodyampalayam coastal village, located in Nagapattinam district, Tamil Nadu, India during December 2009. The specimen identified and deposited in the herbarium of Centre of Advanced Study in Marine Biology Annamalai University, India (Voucher No. AUCASMB 10/2010).

2.3. Extraction and Fractionation

The collected leave materials of *R. apiculata* (3.0 kg) washed with running tap water, shade dried and powdered. The known amount of finely ground plant material extracted with 80% ethanol (5 × 5 L) at room temperature. The ethanolic extract so obtained was freed of solvent under vacuum to get (24.14% yield) of dark greenish-brown mass. The solvent-free extract was acidified by 1M hydrochloric acid further dissolved and extracted with dichloromethane. There are two layers, i.e. dichloromethane and aqueous acid layers were obtained. The dichloromethane layer was separated and allowed to evaporate. The aqueous acid layer was further basified by sodium hydroxide and extracted with ethanol. Ethanolic extract (EE), dichloromethane fraction (DCM-F), and aqueous basic fraction (AB-F) were thus obtained. The qualitative chemical test performed to assess the presence of various phytoconstituent^[18].

2.4. Animals

About 200 to 250 g weight of male albino rats procured from the Central Animal House Facility, Rajah Muthiah Medical College and Hospital (RMMC & H), Annamalai University utilized in this study. The animals were fed on pellet diet (Hindustan Pvt. Ltd., Bangalore, India) water ad libitum. The experimental studies carried out in accordance with the Animal ethical committee of RMMC & H, Annamalai University, India.

2.5. Toxicity Studies

The oral acute toxicity of *R. apiculata* extracts was determined according to OECD-423 guidelines in Swiss albino mice (n=3). The animals were fasted for 4 h with free access to water only. The extract and fraction were administered orally at a dose of five mg/kg initially by suspending in 0.5% CMC solution and mortality observed for three days^[19]. If mortality observed on 2/3 or 3/3 animals, then the dose administered considered as a toxic dose. However, if the mortality observed in only one mouse out of three animals, then the same dose repeated again to confirm the toxic effect. If mortality is not observed, the procedure is repeated with higher doses such as 50, 300, and 2000 mg/kg.

2.6. Effective dose fixation studies

The extract and fractions were orally administered in the dose of 2000 mg/ kg body weight to the animals. The first test animal survived and then four animals subsequently treated. The animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h up to 14 days (daily once). No animal died. Therefore, the lethal dose 50 is greater than 2000 mg/kg^[20]. An investigation with 1/20th, 1/8th and 1/4th of 2000 mg/kg was done in pre-screening. The effective dose 250 mg/kg was used further experiments.

2.7. Induction of Diabetes Mellitus

Insulin-dependent diabetes mellitus induced in rats by the single intraperitoneal injection of STZ (60 mg/kg b.wt.) dissolved in 0.1 mol/L citrate buffer, pH 4.5. The blood glucose level was checked before and 72 h after STZ injection to confirm the development of diabetes. Only those animals which showed hyperglycemia (blood-glucose glucose level > 250 mg/dl) were used in the experiment. NIDDM induced using overnight fasting Wistar rats by a single-dose injection of STZ (60 mg/kg b.wt. I.P.), 15 min after the rats injection of (I.P., 120 mg/kg b.wt.) nicotinamide. NIDDM ensured by the rats was given glucose tolerance test, and fasting insulin resistance index (FIRI) was compared control, and NIDDM control rats. FIRI was calculated via blood sampling from ocular sinus and measurements of fasting insulin and glucose levels.

2.8. Glucose Tolerance Test

NIDDM confirmed rats undergone 12 h fasting, and then 40% glucose solution with the concentration of (2 g/kg of body weight) was intraperitoneally injected to rats. The blood-glucose level was measured at 30, 60, and 120 min after glucose injection. FIRI was calculated by the following formula^[21]

$$\text{FIRI} = \frac{\text{Fasting Insulin (Miu/MI)} \times \text{Fasting Glucose (Mg/Dl)}}{25}$$

2.9. Experimental Design

Overnight fasted normoglycemic and diabetic rats were divided into seven groups with seven rats each. Extracts and fractions suspended in the vehicle (0.5% CMC) and orally administered for 21 days (daily once) as follows,

- Group 1: Normal rats + 0.5% CMC
- Group 2: IDDM control + 0.5% CMC
- Group 3: NIDDM control + 0.5% CMC
- Group 4: IDDM rats + EE (250 mg/kg body weight)
- Group 5: IDDM rats + DCM-F (250 mg/kg body weight)
- Group 6: IDDM rats + AB-F (250 mg/kg body weight)
- Group 7: NIDDM rats + EE (250 mg/kg body weight)
- Group 8: NIDDM rats + DCM-F (250 mg/kg body weight)
- Group 9: NIDDM rats + AB-F (250 mg/kg body weight)

The body weight of rats was noticed on the first and last day of the experiment. On 0th, 7th, 14th, and 21st day, fasting blood glucose estimated by the glucose oxidase – peroxidase method^[22]. Serum cholesterol, triglycerides, and HDL-C were also evaluated in normal and diabetic rats by an autoanalyzer using diagnostic kits according to the manufacturer's instruction (Asritha Diotech, Hyderabad, India).

2.10. Statistical Analysis

All the measurements performed in triplicates, and the data are expressed in mean ± SEM. All the parameters were analyzed using One-way analysis of variance (ANOVA) followed by the Dunnett's Multiple Range Test (DMRT) using Graph Pad Instat software. Comparisons with p-values < 0.05 were considered statistically significant.

3. Result

The qualitative phytochemical investigation revealed the presence of flavonoids, alkaloids, and glycosides in EE.

Furthermore, the DCM-F showed the presence of alkaloids, sterols and AB-F revealed the presence of alkaloids, terpenoids, and glycosides. Acute toxicity studies observed the non-toxic nature of extract and fractions. There was no lethality or any toxic reactions found at any of the doses selected at the end of the study period. The results of the glucose tolerance test showed that fasting blood glucose was significantly different at 30 min and 2 h after glucose injection between control, and NIDDM rats. FIRI also revealed a significant increase in type two diabetic groups compared with control (Fig. 1).

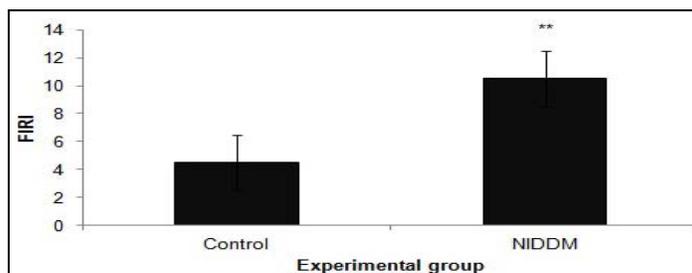


Fig 1: Difference of fasting insulin resistance index between the control and NIDDM rats. **p < 0.01 control vs NIDDM

The present study could complete with 52 rats, and 11 rats died during the execution of the study. STZ induced IDDM in rats with fasting blood sugar level more than 250 mg/dL. Oral administration of DCM-F at 250 mg/kg significantly (P < 0.01) reduced blood glucose level in diabetic rats than compared to EE and AB-F. STZ-nicotinamide induced

NIDDM in rats. Blood glucose level increased in the diabetic control group. Significant reduction (P < 0.01) in the blood glucose levels was brought about in diabetic rats by daily administration of the dichloromethane fractions at 250 mg/kg than compared to the ethanolic extract, and aqueous basic fraction (Table 1).

Table 1: Effect of ethanolic extract, dichloromethane, and acid aqueous fraction on blood glucose level in experimental rats

Group	0 th day	7 th day	14 th day	21 st day
Normal	102 ± 56	082 ± 48	125 ± 34	154 ± 39
IDDM C	615 ± 32	945 ± 37	556 ± 13	050 ± 22
NIDDM C	783 ± 56	044 ± 42	753 ± 24	152 ± 31
IDDM + EE (250 mg/kg)	652 ± 34	422 ± 71	577 ± 26 ^a	220 ± 22 ^a
IDDM + DCM-F (250 mg/kg)	660 ± 11	402 ± 35	558 ± 17 ^b	133 ± 44 ^b
IDDM + AB-F (250 mg/kg)	673 ± 33	435 ± 11	581 ± 22 ^a	244 ± 22 ^a
NIDDM + EE (250 mg/kg)	745 ± 37	555 ± 34	523 ± 34 ^c	193 ± 34 ^c
NIDDM + DCM-F (250 mg/kg)	892 ± 56	632 ± 34	974 ± 41 ^d	385 ± 42 ^d
NIDDM + AB-F (250 mg/kg)	853 ± 32	685 ± 24	422 ± 26 ^c	222 ± 45 ^c

Values are ^a P < 0.05, ^b P < 0.01 vs IDDM; ^c P < 0.05, ^d P < 0.01 vs NIDDM

Treatment with DCM-F caused significant improvement in the HDL-C level as compared with the diabetic control groups. Serum cholesterol and triglyceride levels were decreased significantly (P < 0.01) by DCM-F in IDDM and NIDDM rats (Table 2).

Table 2: Effect of ethanolic extract, dichloromethane, and acid aqueous fraction on serum lipid profile in experimental rats on 21st day

Group	TC	TG	HDL
Normal	872 ± 36	824 ± 45	373 ± 26
IDDM C	547 ± 76	505 ± 51	282 ± 22
NIDDM C	575 ± 54	564 ± 32	243 ± 25
IDDM + EE (250 mg/kg)	185 ± 49 ^a	048 ± 25 ^a	321 ± 38 ^a
IDDM + DCM-F (250 mg/kg)	568 ± 23 ^b	483 ± 47 ^b	211 ± 52 ^b
IDDM + AB-F (250 mg/kg)	132 ± 43 ^a	984 ± 32 ^a	354 ± 32 ^a
NIDDM + EE (250 mg/kg)	220 ± 14 ^c	055 ± 21 ^c	302 ± 20 ^c
NIDDM + DCM-F (250 mg/kg)	174 ± 49 ^d	023 ± 22 ^d	344 ± 43 ^d
NIDDM + AB-F (250 mg/kg)	274 ± 33 ^c	072 ± 41 ^c	303 ± 39 ^c

^a P < 0.05, ^b P < 0.01 vs IDDM; ^c P < 0.05, ^d P < 0.01 vs NIDDM

4. Discussion

STZ is diabetogenic agent destroys the insulin producing β-cells by inducing necrosis. The structural similarity between STZ and glucose, which enables STZ, is enter into the cell through glucose transporter 2 in the plasma membrane [23]. STZ alone induces IDDM with the symptoms include severe glycaemia, glucosuria, polyphagia, polydipsia, and body weight loss, which occur chiefly because of loss of β-cells [24]. In the present study, the hyperglycemic state and body weight loss in the experimental rats indicates STZ destroyed β-cells which results in the induction of insulin dependent diabetes mellitus. Numerous studies performed to understand the pathogenesis of diabetes mellitus. However, complex pathology of human noninsulin dependent diabetes still unknown. Nicotinamide a water-soluble vitamin plays a beneficial role in delaying the onset of IDDM in non-obese diabetic mice [25]. Treatment of prediabetic with nicotinamide induces a diabetic with stable metabolic alterations and reduction in pancreatic insulin [26]. In the present study, we used

STZ/nicotinamide diabetic rat model, with abnormal glucose tolerance and fasting insulin resistance index to confirm noninsulin dependent diabetes mellitus in rats.

Our results show that the ethanolic extract and dichloromethane fraction of *R. apiculata* demonstrated antihyperglycemic effects in IDDM rats. The inactivity of the extract may indicate that the extract also act by stimulation of the islet cells and thus requires functional β -cells for its action. In the NIDDM diabetic model rats, the ethanolic extract and dichloromethane fraction of *R. apiculata* showed an effective anti-hyperglycemic effect comparable to that of the aqueous fraction. Thus, indicates that the extract may act on β -cells like sulfonylurea drugs to stimulate insulin secretion. There are some similar results have been reported with *A. occidentale* aqueous leaf extract^[27].

The dichloromethane and ethanolic extract of *R. apiculata* did not show any hypoglycemic effect in NIDDM rats on fasting condition, it can be assumed that this extract may stimulate insulin secretion in a glucose-dependent manner. On the other hand, the hypoglycemic effect of the extract in rats indicates an inhibition of intestinal glucose absorption and the stimulation of the glucagon-like peptide (GLP-1) which is also a glucose-dependent insulin secretagogue^[28].

Dyslipidemia is one of the complications during diabetic mellitus, the serum lipid profile of rats was evaluated in this study. The untreated diabetic rats expected to show a significant increase in serum TC and TG concentrations against low levels of HDL-C^[29]. This increase in serum lipids is mainly due to the increased fatty acid mobilization from adipose tissue. Since insulin has an inhibitory action on 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, the key enzyme in cholesterol biosynthesis, insulin deficiency, or insulin resistance may therefore be responsible for hyperlipidemia^[30]. Treatment of NIDDM rats with *R. apiculata* extracts, reversed although not completely dyslipidemia as evidenced by the significant decrease in TC, and TG coupled to the increase in HDL-C. These alleviating effects clearly denote the antihyperlipidemic potential of *R. apiculata*.

5. Conclusion

In this study, dichloromethane fractions obtained from the ethanol extract produced important hypoglycemic effects in NIDDM rats, indicating that the antihyperglycemic metabolites of the *R. apiculata* are concentrated in this fraction. However, the nature of the active metabolite responsible this therapeutic effect requires further purification studies. Further purifications and evaluations studies are in progress to elucidate in detail the active principles and the real mechanism of action of this *R. apiculata* extract.

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7. Conflict of Interest

All authors declared there is no conflict of interest.

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