

## Positive Regulation of *Rhizophora mucronata* Poir Extracts on Blood Glucose and Lipid Profile in Diabetic Rats

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### Abstract

**Background:** About, five percent of the population in the world has been clinically determined to have diabetes mellitus and considered as one of the principal reasons for death. The present research designed to assess the impact of *Rhizophora mucronata* leaves ethanolic extract (EtOH-Et) and its dichloromethane (DCM) and aqueous (Aq) fractions in diabetic rats.

**Methods:** Extract and fractions of *R. mucronata* were determined qualitative and quantitative methods. Different dose (50, 100 and 200 mg/kg of body weight) of extract and fractions were administered intraperitoneally (I.P.) to the normal glycemic rats and their hypoglycemic effect determined for 24 h. Type 1 diabetes mellitus (T1DM) was induced by single injection of streptozotocin (STZ; 60 mg/kg; I.P.), and type 2 diabetes mellitus (T2DM) was induced by STZ (60 mg/kg; I.P.), after 15 min nicotinamide (120 mg/kg; I.P.). The optimum dose of 100 mg/kg body weight of extract and fractions was administrated to the rats orally for 14 days. The blood glucose level estimated on 0<sup>th</sup>, 7<sup>th</sup>, and 14<sup>th</sup> day. The level of serum cholesterol, triglycerides, and lipoprotein cholesterol were also evaluated on the 14<sup>th</sup> day.

**Results:** The phytochemical results indicated alkaloids, flavonoids, and terpenoids present in the concentrates of *R. mucronata*. The content of alkaloids in DCM-F was present in high amount compared with ethanolic extract and an aqueous fraction. All the extract exhibited as non-toxic nature. DCM-F treated rats significantly reduced in blood glucose level ( $P < 0.01$ ), 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 of T1DM and T2DM.

**Conclusion:** DCM-F of *R. mucronata* act as effective anti-hyperglycaemic and anti-hyperlipidemic agent in insulin dependent and non-insulin dependent diabetic rats.

**Keywords:** Acute toxicity; Diabetes mellitus; Dichloromethane; Mangrove; Total cholesterol

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### Introduction

Inadequate release of insulin or inconsistent action of insulin results in the prolonged level of blood glucose prompts diabetes mellitus [1]. About 2.5-7% of the population throughout the world has been diagnosed with T2DM and considered as one of the foremost causes of death [2]. Malfunction of essential body organs like eyes, heart, kidney, nerves, and blood vessels is a part

of the major risk factors in the case of chronic hyperglycemia. Several studies have elaborated the factors responsible for T2DM, and the adverse effect of the clinically available drug [3]. However, the usage of medicinal plants extracts and their metabolites as antidiabetic agents still is increasing in the developing countries because of the side effects of the commercial drugs. The mangrove species *R. mucronata* Poir (family: Rhizophoraceae) used as a traditional medicine in the coastal villages of Cuddalore district

in India [4]. Studies reported that *R. mucronata* extracts had a hypoglycemic, anti-radical, anti-cancer, anti-nociceptive, anti-plasmodial, anti-HIV, Peroxisome proliferator activated receptor- $\gamma$  antagonistic effect,  $\alpha$ -amylase along with  $\alpha$ -glucosidase inhibitory effect [5-11]. The fruit flour of *R. mucronata* was used as function food for anti-diabetic effect [12]. The alkaloid fractions of *R. mucronata* leaves have been reported with anti-microbial and radical scavenging effects [13]. There are a number of terpenoids, volatile derivatives, and phenolic constituents identified from the different extracts of this plant [14-16]. The computational studies expressed that alkaloids of *R. mucronata* efficiently interacted with dipeptidyl peptidase-4 receptor (DPP-IV) receptor, alpha-ketoglutarate-dependent dioxygenase, and cyclooxygenase II receptors and inhibit their mode of action [17-19]. Therefore, we hypothesized that the ethanolic extract and its fractions of *R. mucronata* as competent to control blood glucose, and this was evaluated in the diabetic animal model.

## Materials and Methods

### Chemicals and drug

Streptozotocin (STZ) and nicotinamide (NAD) were purchased from Sigma, St. Louis, USA. Colchicine, Quercetin, Gallic acid and other experimental chemicals used were of analytical grade (Hi-media, Mumbai, India). The diagnostic kits for triglycerides, total cholesterol, and high-density lipoprotein C (HDL-C) were obtained from Asritha Diotech, Hyderabad, India.

### Test animals

Male, albino Wistar rats, weighing 200-250 g were procured from the Central Animal House Facility, Rajah Muthiah Medical College and Hospital (RMMC & H), Annamalai University utilized in this study. Animals were fed on a pellet diet (Hindustan Pvt. Ltd., India) and water ad libitum. The animal experiments were followed by the guidelines of National Institute of Health and approved by RMMC&H, India.

### Plant material

During post monsoon season in 2010, the fresh leaves of *R. mucronata* were collected from the Kodiampalayam, Nagapattinam district, Tamil Nadu, India. The plant material was deposited (Voucher no. AUCASMB11/2010) and authenticated at molecular level (GenBank Accession No.: JQ042819; JN008864).

**Extraction and fractionation of *R. mucronata* leaves:** The collected leave materials of *R. mucronata* (1.5 kg) were washed under running tap water, shade dried and powdered. The known amount of powdered material was extracted five times with 5x5 L of ethanol (80%) at 28°C. The solvent was removed under vacuum to get a dark greenish-brown mass of ethanolic extract (27.52 g). This extract was acidified with 1M of hydrochloric acid (HCl) and was then dissolved and fractionated with dichloromethane. Two layers, namely DCM and aqueous acid layers were obtained. The DCM layer was separated and allowed to evaporate. The sedimentary acid layer was further basified by sodium hydroxide and extracted with ethanol. EtOH-Et, DCM-F, and Aq-F were obtained. The qualitative chemical test was performed to determine the phyto-constituents present in the extract and fractions [20].

**Estimation of total alkaloids:** The total alkaloid content of extract and fractions of *R. mucronata* was estimated according to the bromocresol green complex formation method [21]. All experiments performed thrice. Colchicine used as a standard. Results were expressed as mg of colchicine equivalent per gram of dry weight (mg colchicine/g) of extracts.

**Estimation of total phenols and flavonoids:** Total phenols of the extract and fractions of *R. mucronata* were estimated using Folin-Ciocalteu's reagent. Gallic acid used as a standard. The total phenolic content is here expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight of extract [22]. Total flavonoid content was determined using aluminum chloride ( $AlCl_3$ ) and quercetin as a standard. The results expressed as milligram of quercetin equivalent per gram of dry weight (mg quercetin/g) of extracts [23].

**Lethal dose 50 ( $LD_{50}$ ) of *R. mucronata* extract and fractions:** Lethal dose 50 ( $LD_{50}$ ) of *R. mucronata* extract and fractions was performed according to the organization for economic co-operation and development (OECD)-423 guidelines [24]. Overnight fasted mice of both sexes were treated with three different doses of 50, 300, and 2000 (mg/kg b.wt.) of EtOH-Et, DCM-F, and Aq-F, through oral route respectively. The control mice received water alone. The animals were observed for first 72 h and up to seven days for any signs of behavioral changes, toxicity effect, mortality, and body weight. The number of mice died within 24 h was noted, and their lethal dose 50 ( $LD_{50}$ ) of the extract and fraction was calculated.

**Administration of extracts and fractions to normal rats for hypoglycemic studies:** The studies were carried out to determine the optimum dose of ethanolic extract, DCM-F, and Aq-F which exhibited maximal hypoglycemic activity. Rats were randomly divided into ten groups (n=6) with normal control (received only water). Rats treated with the graded dose of EtOH-Et, DCM-F, and Aq-F (50, 100 and 200 mg/kg of b.wt.) in normal saline by intraperitoneal injection. The food was withdrawn during the 6<sup>th</sup> h of the experiment. After determination of blood glucose level at 6<sup>th</sup> h, the food was given to the animals and blood glucose was measured again at 24<sup>th</sup> h using One-Touch Select Simple (Johnson & Johnson Pvt. Ltd. Mumbai, India). From the data obtained, the optimum dosage was fixed as (100 mg/kg b.wt.) for further studies.

**Preparation of diabetic animal models:** T1DM induced in rats by the single injection of STZ (60 mg/kg b.wt.; I.P.) dissolved in 0.1 mol/L citrate buffer, pH 4.5. After 72 h, rats with >250 mg/dl blood glucose confirmed diabetes (blood glucose level) was used in the study. T2DM induced using overnight fasting rats by a single-dose injection of STZ (60 mg/kg b.wt.; I.P.), 15 min after the rats injection of nicotinamide (I.P., 120 mg/kg b.wt.). T2DM ensured by the rats was given glucose tolerance test, and fasting insulin resistance index (FIRI) was compared control, and T2DM control rats. FIRI was calculated *via* blood sampling from ocular sinus and measurements of fasting insulin and glucose levels. T2DM confirmed rats undergone 12 h fasting, and then 40% glucose solution with the concentration of (2 g/kg of b.wt.; I.P.) was injected to rats. After 30, 60, and 120 min of after glucose injection the blood-glucose level was measured [25].

**Administration of extract and fractions to the diabetic animal models:** Overnight fasted diabetic rats were divided into eight groups of seven rats each. Extracts and fractions were orally administered for 14 days (daily once) are mentioned in **Table 1**.

On the first and last day of the experiment, the body weight of rats was noted. On 0<sup>th</sup>, 7<sup>th</sup>, and 14<sup>th</sup>-day fasting blood glucose estimated by the glucose oxidase-peroxidase method [26]. According to the kit manufacturer's instruction serum cholesterol, triglycerides, and HDL-C were also evaluated in normal and diabetic rats.

### Statistical analysis

The data was calculated in mean  $\pm$  standard error mean. 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 4.0.

## Results

### Phytochemical studies

Phytochemical studies indicated phenolics, saponin terpenoids,

**Table 1** Administration of extract and fractions to the diabetic animal models.

No. of group	Treatment
Group 1	Normal rats+0.5 % CMC
Group 2	T1DM control+0.5 % CMC
Group 3	T2DM control+0.5 % CMC
Group 4	T1DM rats+EtOH-Et (100 mg/kg b.wt.) suspended in 0.5% CMC
Group 5	T1DM rats+DCM-F (100 mg/kg b.wt.) suspended in 0.5% CMC
Group 6	T1DM rats+Aq-F (100 mg/kg b.wt.) suspended in 0.5% CMC
Group 7	T2DM rats+EtOH-Et (100 mg/kg b.wt.) suspended in 0.5% CMC
Group 8	T2DM rats+DCM-F (100 mg/kg b.wt.) suspended in 0.5% CMC
Group 9	T2DM rats+Aq-F (100 mg/kg b.wt.) suspended in 0.5% CMC

**Table 2** Qualitative phytochemical screening of ethanolic extract, dichloromethane and aqueous fraction of *R. mucronata*.

Phytochemicals	Tests	Interference	EtOH	DCM-F	Aq-F
Alkaloids	Mayer's reagent	Formation of yellow cream (p)	+++	++++	+
	Wagner's reagent	Formation of reddish brown (p)	++	+++	++
Carbohydrates	Molish's reagent	Formation of violet ring	+	+	+
	Benedict's reagent	Formation of orange red (p)	+	+	+
	Fehling's reagent	Formation of red (p)	+	+	+
Saponin	Foam	Produce foam	-	-	-
Glycosides	Brontrager's	Formation of rose pink colour	+	+	+
Fats and Oil	Filter paper press	Formation of oily stain	+	+	+
Resins	Acetone water	Appearance of turbidity	-	-	-
Phenols	Ferric chloride	Formation of bluish black	++	++	+
Tannins	Gelatin	Formation of white (p)	+	+	+
Diterpenes	Copper acetate	Formation of bright green colour	+	+	++
	Alkaline reagent	Formation of yellow colour	++	+	+
Flavonoids	Lead acetate	Formation of yellow colour	+	+	+
	Shinoda	Formation of magenta colour	+	+	+
	Zinc HCl	Formation of magenta colour	+	+	+
Proteins	Xanthoproteic	Formation of yellow colour	++	++	++

++++: high content; +++: medium content; ++/+ : low content; -: no content (content was evaluated as the sediment or the intensity of colour).

and flavonoids were present in the EtOH extract and the aqueous fraction of *R. mucronata*. DCM-F exhibited the presence of alkaloids in a high amount based on the sedimentation when compared to other phyto-constituents like carbohydrates, diterpenes, flavonoids, fats and oil, glycosides, phenols, proteins, resins, saponin and tannins (**Table 2**). Total alkaloids, flavonoids and phenols in the ethanolic extract, DCM-F and Aq-F were shown in **Table 3**.

### Effect of *R. mucronata* extracts and fractions in the changes of behavior and appearance

The impact of ethanolic extract, dichloromethane and an aqueous portion of *R. mucronata* on the physical and the behavioral changes of mice are appeared in **Table 4**. There were no mortality or any indications of poisonous quality observed in animals, up to seven days. The behavioral changes of animals were seen after the sixth hour (first day), third day and followed by seventh day after the administration of test samples treated animals were normal and did not show noteworthy changes in conduct, skin impacts, breathing, hindrance in nourishment admission and water utilization, postural irregularities and balding. There were no noteworthy changes in body weight. All the animals showed a normal growth in body weight without major difference between both control and treated animals. The toxicity study confirmed that LD<sub>50</sub> value of EtOH, DCM-F, and Aq-F was more than 2000 mg/kg of animal body weight.

### Hypoglycemic activity of EtOH extracts and its fractions of *R. mucronata*

Normoglycemic rats were used in order to evaluate the ability of the extract and fractions of *R. mucronata* in applying a

hypoglycemic effect. The extract and fractions were seen to exhibit moderate hypoglycemic activity (**Figure 1**). 100 mg/kg dose of the test samples exhibited the maximum glucose-lowering potential at the time interval of 2 h following extract administration when compared with other higher doses. It is reducing the blood glucose level by 20% as compared with the control group. Overall, it was observed that the response elicited was not dose-dependent and that the dose of 100 mg/kg was the most effective, displaying a consistent blood glucose lowering effect on normoglycemic rats. Rats were fed after the 6th h reading and blood glucose again recorded at 24 h in the fed state. This reading showed that the 100 mg/kg b.wt. of *R. mucronata* exerted a prolonged effect on the blood glucose level since rats treated with the EtOH-Et, DCM-F and Aq-F were found to have significantly lower levels of blood glucose than the control. None of the doses studied lowered blood glucose level below 50 mg/dl and, hence, it may be stated that the hypoglycemic effect was moderately mild.

### Anti-hyperglycemic and anti-hyperlipidemic effect

The present study could complete with 52 rats, and 11 rats died during the execution of the study. Blood glucose level increased in

**Table 3** Yield of different extracts and quantification of phytochemicals.

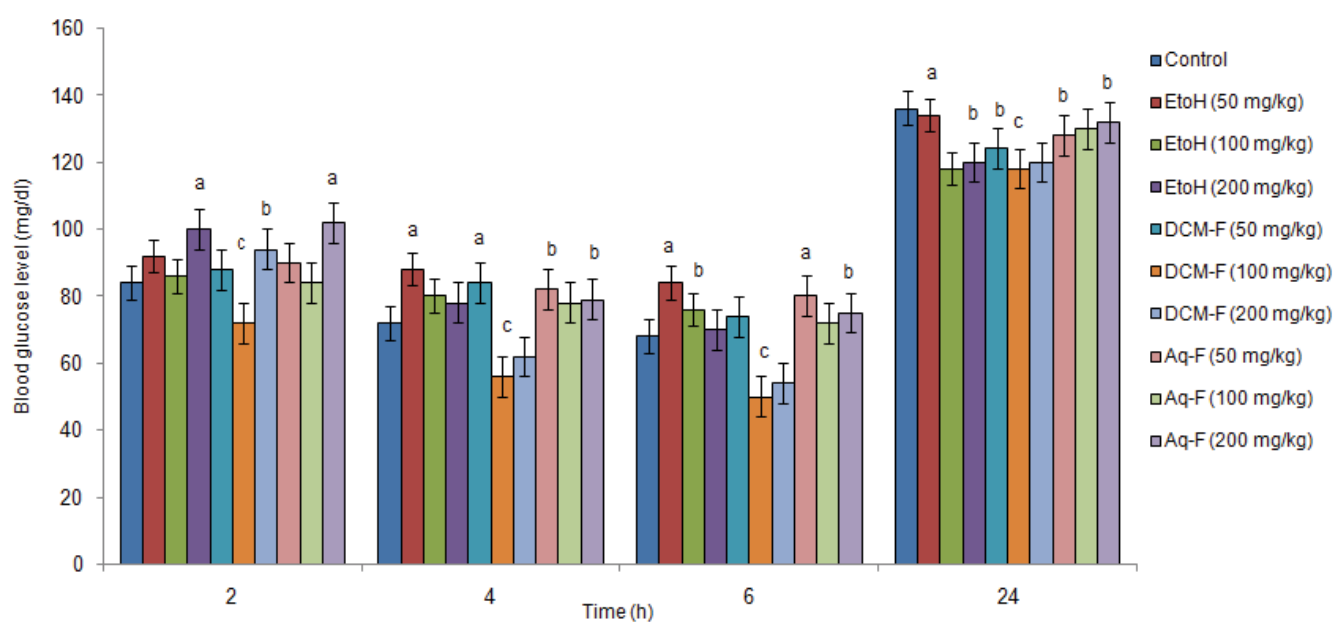
Extracts	Total alkaloids (mg/g)	Total Phenols (mg/g)	Total flavonoids (mg/g)
EtOH-Et	29.34 ± 0.31	30.34 ± 0.74	11.34 ± 0.17
DCM-F	250.53 ± 0.51	190.53 ± 0.15	75.53 ± 0.14
Aq-F	120.42 ± 0.06	95.45 ± 0.05	12.42 ± 0.06

the diabetic control group of both T1DM and T2DM. A significant reduction ( $P < 0.01$ ) in the blood glucose levels was brought about in diabetic rats by daily administration of the DCM-F than compared to the ethanolic extract, and aqueous basic fraction (**Table 5**). 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 T1DM and T2DM rats (**Table 6**).

### Discussion

The ethno-medicinal valuable of mangrove plants had an enormous amount of secondary metabolites when compared to terrestrial plants. The World health organization (WHO) suggested the utilization of plants for the management of diabetes mellitus and encouraged scientific evaluation of its anti-hyperglycemic properties [17,27,28]. In order to explore mangrove plants and maximize the therapeutic benefits, efforts should be geared toward novel anti-diabetic agent development. Therefore, we evaluated *R. mucronata* for possible management of diabetes mellitus. Dichloromethane fractions of different plant extract viz., *Bridelia ndellensis*, *Ficus odorata*, *Rhizophora apiculata* and *Kalanchoe pinnata* [29-31] support the anti-hyperglycemic effect DCM-F of *R. mucronata* in diabetic rats.

Toxicity studies in mice could be used to assess extracts of biological sources for different therapeutic application in a minimum dosage level [32]. It is difficult in definitively evaluating the safety of these test samples are established to have an adequate possible for development into drugs. However, the



**Figure 1** Hypoglycemic effect of ethanolic extract, DCM-F and Aq-F of *R. mucronata*. Values are Mean ± STD. a-  $p < 0.05$ , b-  $p < 0.01$ , c-  $p < 0.001$  compared with control rats.



**Table 4** Behavioural observations and appearance of control and test groups-65432.

Observation	Control group			Test group		
	1 <sup>st</sup> day	3 <sup>rd</sup> day	7 <sup>th</sup> day	1 <sup>st</sup> day	3 <sup>rd</sup> day	7 <sup>th</sup> day
Toxicity at 50 mg/kg	0/3 <sup>a</sup>	0/3	0/3	0/3	0/3	0/3
Toxicity at 300 mg/kg	0/3	0/3	0/3	0/3	1/3	0/3
Toxicity at 2000 mg/kg	0/3	0/3	0/3	0/3	0/3	1/3
Body weight (g)	22.7 ± 0.56	20.7 ± 0.65	21.7 ± 0.79	25.1 ± 0.85	25.7 ± 0.75	24.7 ± 0.25
Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Behavioural patterns	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Lethargy	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhoea	No	No	No	No	No	No
Tremors	No	No	No	No	No	No

<sup>a</sup>=Number of dead mice/ number of mice used; values of body weight are mean ± SD (n=3).

**Table 5** Effect of ethanolic extract, dichloromethane, and acid aqueous fraction on blood glucose level in experimental rats.

Group	0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day
Normal	102 ± 56	082 ± 48	125 ± 34
T1DM C	615 ± 32	945 ± 37	556 ± 13
T2DM C	783 ± 56	044 ± 42	753 ± 24
T1DM+EE (100 mg/kg)	643 ± 29	413 ± 66	564 ± 29 <sup>a</sup>
T1DM+DCM-F (100 mg/kg)	651 ± 13	399 ± 29	547 ± 19 <sup>b</sup>
T1DM+Aq-F (100 mg/kg)	662 ± 26	427 ± 16	573 ± 28 <sup>a</sup>
T2DM+EE (100 mg/kg)	733 ± 31	543 ± 28	512 ± 30 <sup>c</sup>
T2DM+DCM-F (100 mg/kg)	881 ± 45	621 ± 31	467 ± 39 <sup>d</sup>
T2DM+Aq-F (100 mg/kg)	842 ± 29	674 ± 27	493 ± 22 <sup>c</sup>

Values are <sup>a</sup>P<0.05, <sup>b</sup>P<0.01 vs IDDM; <sup>c</sup>P<0.05, <sup>d</sup>P<0.01 vs NIDDM

**Table 6** Effect of ethanolic extract, dichloromethane and acid aqueous fraction on serum lipid profile in experimental rats on 21<sup>st</sup> day.

Group	TC	TG	HDL
Normal	872 ± 36	824 ± 45	373 ± 26
T1DM C	547 ± 76	505 ± 51	282 ± 22
T2DM C	575 ± 54	564 ± 32	243 ± 25
T1DM+EE (100 mg/kg)	174 ± 37 <sup>a</sup>	042 ± 17 <sup>a</sup>	308 ± 33 <sup>a</sup>
T1DM+DCM-F (100 mg/kg)	554 ± 20 <sup>b</sup>	471 ± 41 <sup>b</sup>	201 ± 47 <sup>b</sup>
T1DM+Aq-F (100 mg/kg)	122 ± 39 <sup>a</sup>	973 ± 28 <sup>a</sup>	341 ± 28 <sup>a</sup>
T2DM+EE (100 mg/kg)	215 ± 1 <sup>c</sup>	051 ± 20 <sup>c</sup>	306 ± 18 <sup>c</sup>
T2DM+DCM-F (100 mg/kg)	166 ± 43 <sup>d</sup>	023 ± 19 <sup>d</sup>	335 ± 36 <sup>d</sup>
T2DM+Aq-F (100 mg/kg)	268 ± 29 <sup>c</sup>	065 ± 37 <sup>c</sup>	299 ± 31 <sup>c</sup>

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01 vs IDDM; <sup>c</sup>P<0.05, <sup>d</sup>P<0.01 vs NIDDM

effects of the test samples on nervous and respiratory systems should be evaluated prior to human exposure because it is not defined in OECD-423 [33].

Based on the literature information, the oral route administration is the most convenient because of their slow absorption, cost effective and painless to the animals. Since the extract or fractions are administered orally, the animals should fast before taking the dose to avoid the effect of test samples disturbed by food and other factors. Although there is a trouble about animal experimental data to humans, because toxicity results of mice offer a superior

prophecy for human LD<sub>50</sub> contrast to rats for long-term studies [34]. Therefore, the present study was particularly designed to investigate the toxicity of ethanolic extract, dichloromethane and an aqueous fraction of *R. mucronata* by using acute oral toxicity analysis. The physical appearances such as eyes, fur and skin were revealed to be normal and slight increases in the body weight indicates that the test samples of *R. mucronata* have an insignificant level of cause on animal growth. The results of food intake and water consumption indicate there was no interruption of test samples of *R. mucronata* in metabolism of carbohydrate, fat and protein [35]. It is exciting to observe that the hyperglycemic state in OGTT down within 2 h after administration of DCM-F in an identical way to that of glibenclamide compared to ethanolic extract and aqueous fraction. Thus, it is rational to presume that DCM-F and glibenclamide might enhance the release of insulin or imitate insulin-like action.

STZ is diabetogenic agent destroys the insulin producing β-cells by inducing necrosis. Due to the structural resemblance of STZ is entering into the pancreatic cell through glucose transporter 2 in the plasma membrane [36]. STZ alone induces T1DM with the symptoms include severe glycemia, glucosuria, polyphagia, polydipsia and body weight loss, which occur chiefly because of loss of β-cells. 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 valuable function in delaying the beginning of T1DM in non-obese animals [37]. Treatment of pre-diabetic with nicotinamide induces a diabetic with stable metabolic alterations and reduction in pancreatic insulin [38]. In the present study, we used STZ/nicotinamide diabetic rat model, with abnormal glucose tolerance and FIRI to verify T2DM in rats. Our results show that the ethanolic extract and dichloromethane fraction of *R. mucronata* demonstrated anti-hyperglycemic effects in T1DM rats. The inactivity of the extract may indicate that the extract also acts by stimulation of the pancreatic cells and it needs the

action of  $\beta$ -cells. In the T2DM diabetic model rats, the ethanolic extract and dichloromethane fraction of *R. mucronata* showed an efficient anti-diabetic activity comparable to that of the aqueous fraction. Thus, indicates that the extract may act on  $\beta$ -cells like sulfonylurea drugs to stimulate insulin secretion. There are some similar results have been reported with *A. occidentale* aqueous leaf extract [39]. The dichloromethane and ethanolic extract of *R. mucronata* did not show any hypoglycemic effect in T2DM 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 [40].

3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGCo-A reductase) is one of the important enzymes involved in cholesterol production. However, the insufficient secretion of insulin cannot inhibit the HMGCo-A reductase as a result, hyperlipidemic condition occurs in diabetes mellitus patients [41]. In the mean time, the uncontrolled diabetic circumstances in experimental rats directly proportional to increase in the total cholesterol, triglycerides and decreases in high-density lipoprotein [42]. In the present study, the T2DM rats treated with dichloromethane

fraction of *R. mucronata* shows significantly decreased level of total cholesterol, triglycerides together with elevated level of high-density lipoprotein cholesterol. It clearly elucidates the anti-diabetic and anti-hyperlipidemic functional context of *R. mucronata*.

## Conclusion

In this study, the DCM-F of the ethanol extract is evidenced for vital hypoglycemic and lipidemic effects in T2DM rats by the active metabolites of the *R. mucronata* in this fraction. However, the further purification and characterization of the metabolites are in progress to give imperative outcome for anti diabetic drug development from the *R. mucronata*.

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## Conflict of Interest

All authors declared there is no conflict of interest.

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