# ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECT OF *RUBUS RACEMOSUS* ON STZ INDUCED DIABETIC RATS

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## Summary

The present study was aimed to evaluate the anti diabetic, antihyperlipidemic potential of *Rubus racemosus* in normal streptozotocin induced diabetic mellitus in albino rats. In normal rats, the methanolic extract of *Rubus racemosus* reduced the raised blood glucose level of hyperglycemic due to glucose load significantly. In STZ rats, treatment with methanolic extract of *Rubus racemosus* and Standard glibenclamide reduced the blood glucose, bilirubin, SGOT, SGPT, ALP levels increased total protein and restored the lipid profile. In addition acute toxicity study revealed that the methanolic extract of the plant may be considered relatively safe.

**Key Words:** Antidiabetic, *Rubus racemosus* SGOT, SGPT, MERR, STZ

## Introduction

Diabetic is a major endocrine disorder affecting most of the world population. Diabetic mellitus is treated in Indian traditional medicine using medicinal plants[1&2]. Prolonged treatment of diabetic mellitus with synthetic hypoglycemic agents and insulin produce side effects. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones[3] *Rubus racemosus* commonly called cheethi in tamil[4] belongs to the family Rosaceace is selected for the present work. It is a decidious shrub with pinnate leaves. It's widely distributed in Niligiri hills and Western Ghats of Tamilnadu. Traditionally Rubus is used for antidiabetic, anticonvulsant, antidiabetic and free radical scavenging activity[5]. Decoction of the root is useful for relaxed bowel and dysenterydisorders[6]. Family Rosaceae is known as folk medicine for treatment to nervous disorders[7]. The literature review revealed no documentation report available regarding the antidiabetic potential of aerial parts. Hence the present work was carried out to evaluate scientifically the antidiabetic potential and lipid profile on aerial parts of *Rubus racemosus*.

## Material and methods

### Plant material

Fresh plants of *Rubus racemosus* were collected from Nilgiri hills, Ooty during the month of August identified and authenticated by Dr.S.Rajan, field botanist, survey of medicinal plants and collection unit department of Ayush.

### Preparation of methanolic extract

The aerial parts of authenticated drug were collected shade dried and powdered coarsely. The coarse powder was extracted with methanol in soxhlet apparatus. The extract was evaporated under pressure until the solvent had been removed.

### Experimental animals

Inbred adult Wistar albino rats weighing 150g-200g were obtained from animal house. They were fed with standard pellet diet and water *adlibitum*. Animals were maintained in a standard animal house. The experiments were designed and conducted according to the ethical guidelines after obtaining the necessary clearance from the committee [Approval No: IAEC/XIII/17/CLBMCP/2007-2008]

### Acute toxicity studies [8]

Acute toxicity study was followed according to OECD guidelines 423 [Acute toxic class method. Adult male wistar rats weighing 150-200 g were used for the study. The starting dose of *Rubus racemosus* was 2000 mg/kg body weight. The dose was administered to overnight fasted rats and food was with held for a further 3-4hrs after administration of the drug and observed for signs of toxicity. The animals were kept under observation for 14 days.

### Induction of diabetes in rats

Inbred adult Wistar albino rats were allowed to fast for 24 hours prior to the induction of diabetic mellitus. Freshly prepared streptozotocin was injected to a batch of normalglycemic albino rats intra peritonially at a dose of 50mg/kg of body weight [9]. After two days of streptozotocin induction, animals with glucose level 200 mg/dl were chosen for the studies.

### Experimental design

### Blood glucose in fasting rats

### Effect of MERR treatment on blood glucose level in normo glycemic rats

The animals were divided into three groups of six rats each,

GROUP1 Animals received normal control [1% SCMC 1ml/100gm/po/b.w of rat].

GROUP 2 Animal received MERR [200mg/kg/po/b.w of rat in 1% SCMC].

GROUP 3 Animal received MERR [400mg/kg/po/b.w of rat in 1% SCMC].

In this study, the entire group of animals was overnight fasted prior to the experimentation and administered with the respective drugs as per the above mentioned dosage schedule. Blood samples were collected before administration of the drugs and at 30, 60, 90 and 120$^{th}$ min after drug administration to determine the blood glucose levels by using electronic glucometer[10].

***MERR – Methanolic Extract *of Rubus racemosus***

**Induced blood glucose level**

**Effect of MERR on blood glucose level on glucose fed hyperglycemic rats**

**Oral glucose tolerance test – [OGTT]**

The animals were divided into four groups of six rats each.

GROUP 1 Animals received glucose solution at a dose of 2gm/kg/p.o

GROUP 2 Animals received glibenclamide 0.5mg/kg and glucose solution at a dose of 2 gm/kg/p.o

GROUP 3 Animals received MERR* 200mg/kg/b.w and glucose solution at a dose of 2 gm/kg/oral

GROUP 4 Animals received MERR 400mg/kg/b.w and glucose solution at a dose of 2 gm/kg/p.o

In this study, the entire group of animals was fasted and treated with above dosage schedule only. The MERR and glibenclamide were administered half an hour before administration of glucose solution. Blood samples were collected before glucose administration and at 30, 60, 90 and 120$^{th}$ min after glucose administration to determine the blood glucose level by using electronic glucometer[11].

**Effect of acute treatment of MERR on blood glucose level in STZ induced diabetic rats**

The animals were divided into five groups. Group 1 consisted of 6 normal animals. The remaining 4 groups consisted of 6 STZ* induced diabetic rats.

GROUP 1 Normal control animals received 1% SCMC 2ml/kg/p.o

GROUP 2 Streptozotocin [50 mg/kg/b.w] induced diabetic animals received 1% scmc 2ml/kg/p.o

GROUP 3 Streptozotocin [50 mg/kg/b.w] induced diabetic animals received gliben clamide 0.5 mg/kg/p.o

GROUP 4 Streptozotocin [50mg/kg/b.w] induced diabetic animals received MERR 200mg/kg/p.o

GROUP 5 Streptozotocin [50 mg/kg/b.w] induced diabetic animals received MERR 400mg/kg/p.o

In the single day acute study all the surviving diabetic animals and normal animals were fasted overnight. Blood samples were collected from the fasted animals prior to the treatment with above dosage schedule and after drug administration at 1st, 3 rd and 5th hour to determine the blood glucose level by using electronic glucometer[12].

*** STZ – Steptozotocin**

***MERR – Methanolic Extract of *Rubus racemosus***

**Effect of sub acute treatment of MERR on blood glucose level in STZ induced diabetic rats**

GROUP1 Normal control animals received 1% SCMC 2ml/kg/p.o for 28 days

GROUP2 Streptozotocin [50 mg/kg/b.w] induced diabetic animals received 1% scmc 2ml/kg/p.o for 28 days

GROUP3 Streptozotocin [50mg/kg/b.w] induced diabetic animals received glibenclamide 0.5 mg/kg/p.o for 28 days

GROUP4 Streptozotocin [50 mg/kg/b.w] induced diabetic animals received MERR 200mg/kg/p.o for 28 days

GROUP5 Streptozotocin [50 mg/kg/b.w] induced diabetic animals received MERR 400mg/kg/p.o for 28 days

The above mentioned treatment schedule was followed for the respective group of animals for 28 days. Blood samples were collected from overnight fasted animals, in morning one hour after drug administration on the 1st, 7th, 14th, 21th and 28th day and the blood glucose levels were estimated using electronic glucometer[13]. At the end of study, all the surviving animals were anaesthetized by anaesthetic ether. Blood was collected by bleeding carotid artery into sterile dry centrifuge tubes and allowed to coagulate for 30 min at $37^0$c. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function. All the enzymes assays were carried out at using Shimadzu spectrophotometer, UV-1601 model[14]. Total bilirubin was estimated using the test kit based on vadeberg method[15]. SGOT, SGPT levels were estimated test kit by Reitman and Frankel method[16]. Total protein was estimated using total protein kit based on biuret method[17]. ALP protein kit based on para nitro phenyl phosphate method[18]. Serum total cholesterol, LDL and HDL cholesterol were estimated using cholesterol oxidase/peroxidase method[19]. Triglycerides were estimate by glycerol phosphate oxidase/ peroxidase method.

**Statistical analysis**

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's 't' test. p values <0.05 were considered as significant.

## Results

In acute oral toxicity studies, there was no considerable weight change in body weight before and after treatment and no signs of toxicity was observed.

Table 1 showed the MERR at a dose level of 200mg and 400mg/kg body weight did not exhibit significant hypoglycemic action at the end of 60, 90 and 120 min after oral administration in the normal rats.

### Effect of MERR treatment on blood glucose level in normoglycaemic rats

**Table 1**

| Groups | Initial | 30min | 60min | 90min | 120min |
|---|---|---|---|---|---|
| **I** | 75.56 ± 0.87 | 87.85 ± 0.59 | 112.05 ± 1.07 | 105.03 ± 0.37 | 97.16 ±0.71 |
| **II** | 76.1 ± 0.47 | 111.8 ± 0.76 | 117.16 ± 0.78 | 85.33 ± 0.77 | 72.73 ± 1.49 |
| **III** | 76.08 ± 0.41 | 95.16 ± 1.05 | 104 ± 0.93 | 91.66 ± 0.64 | 80.8 ± 0.36 |

The values are expressed as mean ± SEM. Each groups having six animals. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test. The blood glucose values of group II and III are compared with control animal values.

Table 2 showed MERR at a dose level 200 , 400mg/kg body weight and glibenclamide reduced the raised blood glucose level [hyperglycemic due to glucose load 2 gm/kg/p.o at 4 ml/kg] significantly ($p<0.001$) after 120 min of oral administration when compared to control group.

### Effect of MERR on blood glucose fed hyperglycaemic rats

**Table 2**

| Groups | Initial | 30min | 60min | 90min | 120min |
|---|---|---|---|---|---|
| **I** | 76.28 ± 0.7 | 74.8 ± 0.64 | 70.71 ± 1.00 | 69.66 ± 0.79 | 72.08 ± 0.69 |
| **II** | 79.71 ± 1.24$^{a***}$ | 126.5 ± 0.84$^{a***}$ | 129 ± 0.52$^{a***}$ | 113.46 ± 1.27$^{a***}$ | 80.33 ± 0.52$^{a***}$ |
| **III** | 77.81 ± 0.79$^{b***}$ | 130 ± 0.93$^{b***}$ | 117.5 ± 1.05$^{b***}$ | 95.66 ± 0.91$^{b***}$ | 82.33 ± 0.60$^{b***}$ |
| **IV** | 80 ± 0.78$^{b***}$ | 138.1 ± 0.52$^{b***}$ | 114.98 ± 0.65$^{b***}$ | 98.7 ± 0.64$^{b***}$ | 88.56 ± 0.8$^{b***}$ |

The values are expressed as mean $\pm$ SEM. Each group having six animals. Statistical significant test for comparison done by ANOVA, followed by Dunnet's "t" test.

(a) Group II is compared with group I values
(b) Group III and IV are compared with group II values.

The $60^{th}$, $90^{th}$ and $120^{th}$ min values are compared with initial values: non significant. ***$p<0.001$

Table 3 showed the acute treatment of the effect of MERR at a dose level of 200 ,400mg/kg/p.o and glibenclamide did not produce significant reduction in the blood glucose levels in STZ induced diabetic rats.

**Effect of acute treatment of MERR on blood glucose in STZ induced diabetic rats**

**Table 3**

| Groups | Initial | 1hr | 3hr | 5hr |
|---|---|---|---|---|
| **I** | 86.28 ± 0.4 | 95.8 ± 0.53 | 95 ± 0.66 | 86.05 ± 0.57 |
| **II** | 274.33 ± 2.26$^{ns}$ | 286 ± 1.07$^{ns}$ | 297.83 ± 0.9$^{ns}$ | 294.1 ± 0.61$^{ns}$ |
| **III** | 270.16 ± 1.66$^{ns}$ | 279 ± 2.18 $^{ns}$ | 273.7 ± 1.94 $^{ns}$ | 256.8 ±0.52$^{ns}$ |
| **IV** | 245.83 ± 0.52$^{ns}$ | 277.67 ± 2.07$^{ns}$ | 250.5 ± 1.50$^{ns}$ | 236.7 ± 0.52$^{ns}$ |
| **V** | 234.33 ± 0.74$^{ns}$ | 247.33 ± 1.38$^{ns}$ | 256 ± 1.30$^{ns}$ | 230 ± 3.34$^{ns}$ |

The values are expressed as mean ± SEM. Each groups having six animals. Statistical significant test for comparison done by ANOVA, followed by Dunnet's "t" test. The values 1$^{st}$, 3$^{rd}$ and 5$^{rd}$ hour are compared with initial values. ns – non significant

Table 4 showed that the sub acute study STZ induced diabetic rats were treated with MERR 200 mg/kg/p.o and 400mg/kg/p.o for duration of 28 days significantly [p<0.001] decreased the blood glucose level after 21 days in diabetic rats. Treatment with MERR produced a significant drop in blood glucose level from 21$^{st}$ day onwards up to 28 days. Treatment with gilbenclamide produced significant [p<0.001] decrease in blood glucose level steadily after the 14$^{th}$ day of oral administration.

**Effect of sub-acute treatment of MERR on blood glucose in STZ induced diabetic rats**

**Table 4**

| Groups | Initial | 1 day | 7 day | 14 day | 21 day | 28 day |
|---|---|---|---|---|---|---|
| **I** | 67.66 ± 0.53 | 75.33 ± 0.84 | 67.9 ± 0.54 | 71.5 ± 0.52 | 67.8 ± 0.37 | 69.33 ± 1.36 |
| **II** | 257.5 ± 0.96 | 277.66 ± 1.05 $^{a***}$ | 297.73 ± 0.63 $^{a***}$ | 334.5 ± 4.06$^{a***}$ | 356.33 ± 1.84 $^{a***}$ | 365.16 ± 1.20$^{a***}$ |
| **III** | 255.16 ± 0.9 | 276.66 ± 0.39$^{b**}$ | 278.83 ± 1.35$^{b**}$ | 242.5 ± 3.28$^{b**}$ | 218.16 ± 1.94$^{b***}$ | 191.66 ± 2.64$^{b***}$ |
| **IV** | 248.66 ± 1.38 | 267.66 ± 0.90$^{b**}$ | 271.26 ± 1.50$^{b**}$ | 255.3 ± 1.12$^{b**}$ | 228.5 ± 1.86$^{b***}$ | 163.16 ± 8.31$^{b***}$ |
| **V** | 248.66 ± 1.08 | 264.83 ± 1.37 $^{b**}$ | 268.66 ± 3.56 $^{b**}$ | 191.66 ± 1.12$^{b***}$ | 168.33 ± 2.02$^{b***}$ | 135.3 ± 1.2$^{b***}$ |

The values are expressed as mean ± SEM. Each groups having six animals. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test.

(a) Group II is compared with group I values

(b) Group III, IV and V are compared with group II values. ***p<0.001;** p<0.01

Table 5 showed MERR and glibenclamide showed significant decrease in bilirubin, SGOT, ALP, LDL cholesterol, triglycerides[p<0.001], SGPT and total cholesterol[p<0.01]. MERR and glibenclamide showed significant[p<0.001] increase in total protein and HDL cholesterol when compared to STZ induced diabetic rats.

**Biochemical Parameters**

**Table 5**

| Group | Treatment | Total Bilirubin (mg/dl) | SGOT (IU/dl) | SGPT (IU/dl) | Total protein (mg/dl) | Alkaline Phosphatase (mg/dl) | Total Cholesterol (mg/dl) | HDL-Cholesterol ( mg/dl) | Triglyceride (mg/dl) | LDL-Cholesterol ( mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|
| I (n=6) | Control | 0.17 ± 0.006 | 36.9 ± 0.46 | 42.8 ± 0.55 | 7.3 ± 0.11 | 68.1 ± 0.31 | 64.46 ± 0.15 | 48.13 ± 0.26 | 43.93 ± 0.26 | 18.6 ± 0.40 |
| II (n=6) | Diabetic Control | 0.38 ± 0.007a*** | 92.57±0 .22a** | 95.36±1. 1a** | 3.55 ± 0.14a*** | 155.9 ± 0.58a*** | 125.5 ± 0.76a*** | 18.85 ± 0.18a*** | 64.9 ± 0.13a*** | 40.13 ± 0.78a*** |
| III (n=6) | Gilbenclamide | 0.25 ± 0.01b*** | 63.46±0 .23b** | 64.18±0. 51b** | 5.75 ± 0.13b*** | 118.2 ± 0.22b*** | 86.88 ± 0.23b*** | 40.37 ± 0.38b*** | 56.9 ± 0.18b*** | 16.35 ± 0.19b*** |
| IV (n=6) | MERR 200mg | 0.19 ± 0.006b*** | 42.01±0 .75b** | 55.58±0. 24b** | 6.65 ± 0.07b*** | 96.1 ± 0.71b*** | 74.75 ± 0.28b*** | 45.2 ± 0.3b*** | 58.1 ± 0.12b*** | 14.55 ± 0.07b*** |
| V (n=6) | MERR 400mg | 0.23 ± 0.004b*** | 45.62±0 .06b*** | 58.35±0. 22b** | 6.16 ±0.03b*** | 104.8 ± 0.34b*** | 68.5 ± 0.32b*** | 46.13 ± 0.13b*** | 55.6 ± 0.11b*** | 13.92 ± 0.09b*** |

The values are expressed as mean ± SEM. Each groups having six animals. n= number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test.

(a) Group II is compared with group I values

(b) Group III, IVand V is compared with group II values. ***p<0.001; ** p<0.01

## Discussion

Acute toxicity studies that MERR is relatively non toxic up to 2000mg/kg body weight indirectly pronouncing the safety profile of the extract. MERR at both doses did not significantly suppress blood glucose level in over fasted normal animals. MERR significantly improved in glucose tolerance in glucose fed hyperglycemic rats. In acute treatment a double dose of MERR did not bring about any hypoglycemic action in streptzotocin induced diabetic rats. In the sub-acute study, glibenclamide and MERR treatment brought down the blood sugar levels from the $14^{th}$ day of treatment and a steady decrease in blood glucose levels from $21^{st}$ day of treatment and a steady decrease were observed thereafter. At the end of the study, a marked anti hyperglycemic effect was observed in the plant extract treatment. Serum lipid levels is usually raised in diabetic conditions and is likely to increase risk of coronary heart disease, the glibenclamide and plant material extract treatment is diabetic animals produced beneficial improvement in the lipid profile by a reduction in the total cholesterol levels and increase in HDL cholesterol level. We conclude that the plant under study *Rubus racemosus* could be effective in blood glucose levels and improve lipid profile in diabetic rats.

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