



## The antihyperglycemic activity of leaves of *Leptadenia reticulata* Wight & Arn. (Goan-kha) on adrenaline-induced hyperglycemic rats

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### Abstract

**Background:** In Myanmar, the leaves of *Leptadenia reticulata* Wight & Arn. (locally known as Goan-kha) are traditionally used for their purported anti-hyperglycemic effects. However, scientific validation of these effects remains limited.

**Objective:** To evaluate the anti-hyperglycemic activity of a 70% ethanolic extract of *Leptadenia reticulata* Wight & Arn. leaves in an adrenaline-induced hyperglycemia model in rats.

**Method:** A laboratory-based experimental study was conducted using thirty Wistar rats (180–220 g), which were randomly assigned into five groups (n = 6 per group): a normal control group (received normal saline, 10 mL/kg), a positive control group (received glibenclamide, 4 mg/kg), and three test groups administered ethanolic extract of *L. reticulata* at doses of 150, 300, and 600 mg/kg body weight, respectively. All animals were fasted for 12 hours prior to treatment. Forty-five minutes after drug or extract administration, hyperglycemia was induced via subcutaneous injection of adrenaline (0.8 mg/kg) in all groups. Blood glucose levels were measured using a glucometer at baseline (fasting), and at 1, 2, 3, and 4 hours post-adrenaline injection.

**Results:** In the control group, mean blood glucose concentrations (mg/dL) at 1, 2, 3, and 4 hours post-adrenaline injection were  $117.17 \pm 20.38$ ,  $316.00 \pm 77.24$ ,  $232.33 \pm 48.36$ ,  $133.33 \pm 21.31$ , and  $106.83 \pm 10.69$ , respectively. Significantly lower blood glucose levels were observed in all extract-treated groups compared to the normal saline group at all time points ( $p < 0.003$ ,  $p < 0.018$ ,  $p < 0.048$ ,  $p < 0.003$ ). An acute toxicity study following OECD Guideline 425 revealed no lethality at doses up to 2000 mg/kg. Phytochemical screening indicated the presence of flavonoids, alkaloids, saponins, tannins, phenols, carbohydrates, amino acids, and resins, whereas glycosides and steroids were absent.

**Conclusion:** The 70% ethanolic leaf extract of *Leptadenia reticulata* Wight & Arn. demonstrated significant short-term anti-hyperglycemic activity in adrenaline-induced hyperglycemic rats without observable toxicity, suggesting its potential as a supportive therapeutic agent for type 2 diabetes mellitus, particularly under stress-related hyperglycemic conditions.

**Keywords:** Acute toxicity; Anti-hyperglycemic activity; Ethanolic extract; *Leptadenia reticulata*; Phytochemicals

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## 1. Introduction

Herbal medicines encompass a range of products, including whole herbs, herbal materials, and prepared formulations, that contain biologically active compounds derived from plants. These substances are utilized in the treatment and management of various diseases and health conditions. A number of traditional herbal remedies have been successfully developed into modern pharmacological agents, such as artemisinin, digoxin, morphine, and colchicine. This underscores the importance of scientific investigation aimed at preserving plant biodiversity, elucidating their pharmacological properties, and safeguarding traditional medicinal knowledge[1]. According to an international monograph, Myanmar, among Southeast Asian countries, contributes significantly to medicinal plant diversity, with documentation of 123 families, 367 genera, and 472 species of medicinal plants from a variety of sources[2].

Diabetes mellitus is a chronic metabolic disorder characterized by inadequate insulin production or insulin resistance at the cellular level, resulting in impaired regulation of blood glucose concentrations. The global burden of diabetes has risen markedly over the past few decades. According to the World Health Organization (WHO), diabetes was the ninth leading cause of mortality in 2019. In addition to its health impact, diabetes imposes substantial economic burdens and is associated with reduced life expectancy for affected individuals and their families[1]. In Myanmar, over 1,282,700 cases of diabetes were reported in 2020[3].

Previous international studies have investigated the pharmacological properties of various parts of *Leptadenia reticulata* Wight & Arn., and have reported a range of therapeutic activities, including anti-cancer[4], anti-bacterial[5], hepatoprotective[6], antioxidant[7], anti-asthmatic [8], anti-diabetic[9], and anti-inflammatory effects[10]. In Myanmar, *Leptadenia reticulata*, locally known as Goan-kha, is traditionally used for its anti-hyperglycemic properties. Ethnobotanical knowledge indicates that decoctions prepared from the root of Goan-kha are commonly utilized as a traditional anti-diabetic remedy, particularly among rural populations in central Myanmar. The plant is widely distributed across this region, where it grows as a shrub and is frequently included in betel quid preparations by local communities[11].

This study aimed to investigate the anti-hyperglycemic activity of the leaf extract of *Leptadenia reticulata* Wight & Arn. (Goan-kha), a plant commonly found in central Myanmar, using an adrenaline-induced hyperglycemic rat model. In addition, the phytochemical constituents of the leaf extract were identified, and its acute toxicity profile was assessed to evaluate its safety for potential therapeutic use.

Preparation of 70% ethanolic extraction of *Leptadenia reticulata* Wight & Arn. (Goan-kha) leaves and phytochemical analysis were done at the Department of Pharmacology, Defence Services Medical Academy, Yangon, Myanmar. Acute toxicity testing and the study of the anti-hyperglycemic activity of the leaf extract of *Leptadenia reticulata* Wight & Arn. (Goan-kha) on adrenaline-induced hyperglycemic rats were done at the Animal Laboratory, Defence Services Medical Research Center, Naypyitaw, Myanmar.

## 2. Methods

### 2.1. Collection of plant materials

The entire plant, including the leaves, roots, flowers, and stems of *Leptadenia reticulata* Wight & Arn. (Goan-kha), was collected during the rainy season from Tatkone Township, Myanmar. Botanical identification and taxonomic classification of the plant specimens were conducted at the Department of Botany, University of Yangon, Myanmar.

### 2.2. Preparation of 70% ethanolic extract of leaves of *Leptadenia reticulata* Wight & Arn.

The collected leaves were air-dried in the shade and subsequently ground into a fine powder, which was stored in airtight containers until further use. A total of 145 g of the powdered *Leptadenia reticulata* Wight & Arn. leaves was transferred to a 2-liter conical flask and macerated with 1 liter of 70% ethanol. The mixture was subjected to reflux extraction on a water bath at 70 °C for 6 hours. Following extraction, the mixture was allowed to cool to room temperature and then filtered through filter paper. The resulting filtrate was poured into Petri dishes and evaporated to dryness on a boiling water bath maintained at 100 °C for 20 hours. A brown, sticky crude extract was obtained. The required quantity of this extract was subsequently emulsified with Tween for use in experimental procedures.

### 2.3. Phytochemical analysis of 70% ethanolic extract of leaves of *Leptadenia reticulata* Wight & Arn.

The ethanolic leaf extract of *Leptadenia reticulata* Wight & Arn. was subjected to qualitative phytochemical screening using standard procedures described by Harborne[12]. The analysis was conducted to detect the presence of various classes of bioactive compounds, including alkaloids,  $\alpha$ -amino acids, carbohydrates, flavonoids, phenolic compounds, cyanogenic glycosides, glycosides, saponins, tannins, steroids, terpenes, and reducing sugars.

### 2.4. Acute toxicity study of 70% ethanolic extract of leaves of *Leptadenia reticulata* Wight and Arn.

The acute toxicity and determination of the median lethal dose ( $LD_{50}$ ) of the *Leptadenia reticulata* Wight & Arn. (Goan-kha) leaf extract was conducted in accordance with the OECD Guideline 425 (Up-and-Down Procedure)[13]. As the extract was intended for oral administration, the acute toxicity study was performed via the oral route.

### 2.5. Experiment for anti-hyperglycemic effect of 70% ethanolic extract of leaves of *Leptadenia reticulata* Wight and Arn.

#### 2.5.1. Animal preparation

Rats were randomly assigned to five groups, with six animals per group. The rats were acclimatized to the laboratory conditions for at least 5 days prior to the experiment. Each group was tested on a separate day. Standardized procedures for blood sample collection and adrenaline injection were applied uniformly across all experimental groups.

#### 2.5.2. Determination of fasting blood glucose level of rats

Rats in all five groups ( $n = 6$ ) were fasted for 12 hours and subsequently placed in a mechanical restraint device. A small part of the tip of the tail (1 mm) was cut off to collect blood samples. One drop of blood was applied to a test strip, and blood glucose concentrations were measured using a glucometer. Fasting blood glucose levels were recorded at 8 a.m.

#### 2.5.3. Determination of anti-hyperglycaemic effect on adrenalin-induced hyperglycaemic rats

Fasting blood glucose levels of all rats in each group were measured using the previously described method. Following fasting blood glucose measurement, rats in the negative control group received normal saline (10 mL/kg), rats in the positive control group were administered glibenclamide (4 mg/kg), and three experimental groups received varying doses of the 70% ethanolic leaf extract of *Leptadenia reticulata* Wight & Arn. (150 mg/kg body weight, 300 mg/kg body weight, and 600 mg/kg body weight). After a 45-minute period following drug administration, all rats were injected subcutaneously with adrenaline (0.8 mg/kg body weight). Blood samples were then collected from the tail at 1, 2, 3, and 4 hours post-adrenaline injection, and blood glucose levels were measured using a glucometer.

### 2.6. Data analysis

The data were analyzed by using SPSS software version 25. The blood glucose concentrations following adrenaline injection in all test groups were compared with control by using one-way ANOVA. P value < 0.05 was used to indicate statistical significance.

## 3. Results

### 3.1. Phytochemical analysis of leaves of *Leptadenia reticulata* Wight & Arn.

The leaves of Goan-kha contain flavonoids, alkaloids, saponins, tannins, phenols, carbohydrates, amino acids, and resins; however, glycosides and steroids were not detected.

### 3.2. Result of acute toxicity study

Following oral administration of the 70% ethanolic leaf extract of *Leptadenia reticulata* Wight & Arn., no severe toxic signs or lethality were observed up to a dose of 2000 mg/kg. Thus, the extract was determined to be free from acute toxicity or harmful effects at doses up to 2000 mg/kg.

### 3.3. Results of anti-hyperglycemic effect 70% of ethanolic extract of *Leptadenia reticulata* Wight & Arn. on the adrenaline-induced hyperglycemic rats

The mean blood glucose levels of six albino rats in the normal saline (negative control) group were measured at fasting, and at 1, 2, 3, and 4 hours following subcutaneous injection of adrenaline (0.8 mg/kg). The results were as follows:

117.17  $\pm$  8.32 mg/dL (fasting), 316  $\pm$  31.53 mg/dL (1 hour), 232.33  $\pm$  19.74 mg/dL (2 hours), 133.33  $\pm$  8.70 mg/dL (3 hours), and 106.83  $\pm$  10.69 mg/dL (4 hours). These results are presented in Table 1.

The mean blood glucose levels of the positive control group, which received glibenclamide (4 mg/kg), were measured at fasting, and at 1, 2, 3, and 4 hours following subcutaneous injection of adrenaline (0.8 mg/kg). The results were as follows: 115.67  $\pm$  7.45 mg/dL (fasting), 224.50  $\pm$  11.31 mg/dL (1 hour), 187.00  $\pm$  7.43 mg/dL (2 hours), 92.17  $\pm$  12.71 mg/dL (3 hours), and 67.83  $\pm$  7.01 mg/dL (4 hours). These results are presented in Table 2.

The mean blood glucose levels of test group I, which was treated with the ethanolic extract of *Leptadenia reticulata* Wight & Arn. (150 mg/kg body weight), were measured at fasting, and at 1, 2, 3, and 4 hours following subcutaneous injection of adrenaline (0.8 mg/kg). The results were as follows: 133  $\pm$  5.11 mg/dL (fasting), 247.33  $\pm$  6.27 mg/dL (1 hour), 187.83  $\pm$  5.54 mg/dL (2 hours), 104.67  $\pm$  22.83 mg/dL (3 hours), and 77.17  $\pm$  6.62 mg/dL (4 hours). These results are presented in Table 3.

The mean blood glucose levels of test group II, which was treated with the ethanolic extract of *Leptadenia reticulata* Wight & Arn. (300 mg/kg body weight), were measured at fasting, and at 1, 2, 3, and 4 hours following subcutaneous injection of adrenaline (0.8 mg/kg). The results were as follows: 126.17  $\pm$  4.63 mg/dL (fasting), 243.33  $\pm$  5.20 mg/dL (1 hour), 171.83  $\pm$  15.89 mg/dL (2 hours), 107.33  $\pm$  7.85 mg/dL (3 hours), and 76.17  $\pm$  2.99 mg/dL (4 hours). These results are presented in Table 4.

The mean blood glucose levels of test group III, which was treated with the ethanolic extract of *Leptadenia reticulata* Wight & Arn. (600 mg/kg body weight), were measured at fasting, and at 1, 2, 3, and 4 hours following subcutaneous injection of adrenaline (0.8 mg/kg). The results were as follows: 121  $\pm$  4.26 mg/dL (fasting), 233.50  $\pm$  6.67 mg/dL (1 hour), 152  $\pm$  5.74 mg/dL (2 hours), 104.33  $\pm$  6.19 mg/dL (3 hours), and 71.33  $\pm$  2.38 mg/dL (4 hours). These results are presented in Table 5.

A comparison of blood glucose concentrations between the normal saline group, positive control group (glibenclamide), and test groups I (ethanolic extract 150 mg/kg), II (ethanolic extract 300 mg/kg), and III (ethanolic extract 600 mg/kg) is presented in Table 6. Significantly lower blood glucose concentrations following adrenaline injection were observed in all test groups when compared with the normal saline group ( $p < 0.003$ ,  $p < 0.018$ ,  $p < 0.048$ ,  $p < 0.003$ ), as determined by one-way ANOVA.

**Table 1** Blood glucose concentration of rats fasting and after subcutaneous injection of adrenaline 0.8 mg/kg (normal saline group)

| Rat no.       | Blood glucose concentration (mg/dL) |                    |                    |                    |                    |
|---------------|-------------------------------------|--------------------|--------------------|--------------------|--------------------|
|               | Fasting                             | 1hr                | 2hr                | 3hr                | 4hr                |
| 1             | 146                                 | 406                | 301                | 170                | 155                |
| 2             | 137                                 | 353                | 242                | 133                | 99                 |
| 3             | 92                                  | 392                | 276                | 140                | 109                |
| 4             | 114                                 | 270                | 189                | 120                | 80                 |
| 5             | 108                                 | 229                | 191                | 130                | 89                 |
| 6             | 106                                 | 246                | 195                | 107                | 109                |
| sum           | 636                                 | 1896               | 1394               | 800                | 641                |
| Mean $\pm$ SD | 117.17 $\pm$ 20.38                  | 316.00 $\pm$ 77.24 | 232.33 $\pm$ 48.36 | 133.33 $\pm$ 21.31 | 106.83 $\pm$ 26.19 |
| Mean $\pm$ SE | 117.17 $\pm$ 8.32                   | 316 $\pm$ 31.53    | 232.33 $\pm$ 19.74 | 133.33 $\pm$ 8.70  | 106.83 $\pm$ 10.69 |

**Table 2** Blood glucose concentration of rats fasting and after subcutaneous injection of glibenclamide 4 mg/kg (positive control group)

| Rat code      | Blood glucose concentration (mg/dL) |                    |                    |                   |                   |
|---------------|-------------------------------------|--------------------|--------------------|-------------------|-------------------|
|               | Fasting                             | 1hr                | 2hr                | 3hr               | 4hr               |
| 1             | 110                                 | 260                | 211                | 110               | 80                |
| 2             | 116                                 | 251                | 201                | 137               | 90                |
| 3             | 104                                 | 234                | 197                | 110               | 79                |
| 4             | 90                                  | 198                | 168                | 68                | 50                |
| 5             | 103                                 | 208                | 171                | 60                | 52                |
| 6             | 115                                 | 196                | 174                | 68                | 56                |
| Sum           | 638                                 | 1347               | 1122               | 553               | 407               |
| Mean $\pm$ SD | 115.67 $\pm$ 18.25                  | 224.50 $\pm$ 27.71 | 187.00 $\pm$ 18.21 | 92.17 $\pm$ 31.14 | 67.83 $\pm$ 17.16 |
| Mean $\pm$ SE | 115.67 $\pm$ 7.45                   | 224.50 $\pm$ 11.31 | 187.00 $\pm$ 7.43  | 92.17 $\pm$ 12.71 | 67.83 $\pm$ 7.01  |

**Table 3** Anti-hyperglycemic effects of 70% ethanolic extract of leaves of *Leptadenia reticulata* Wight & Arn. 150 mg/kg bw in adrenaline-induced hyperglycemic rats (group I)

| Rat code      | Blood glucose concentration (mg/dL) |                    |                    |                    |                   |
|---------------|-------------------------------------|--------------------|--------------------|--------------------|-------------------|
|               | Fasting                             | 1hr                | 2hr                | 3hr                | 4hr               |
| 1             | 107                                 | 217                | 178                | 121                | 89                |
| 2             | 102                                 | 258                | 202                | 137                | 98                |
| 3             | 98                                  | 257                | 207                | 111                | 87                |
| 4             | 114                                 | 254                | 186                | 98                 | 68                |
| 5             | 116                                 | 248                | 174                | 85                 | 62                |
| 6             | 100                                 | 250                | 180                | 76                 | 59                |
| Sum           | 637                                 | 1484               | 1127               | 628                | 463               |
| Mean $\pm$ SD | 133 $\pm$ 12.51                     | 247.33 $\pm$ 15.36 | 187.83 $\pm$ 13.57 | 104.67 $\pm$ 22.83 | 77.17 $\pm$ 16.22 |
| Mean $\pm$ SE | 133 $\pm$ 5.11                      | 247.33 $\pm$ 6.27  | 187.83 $\pm$ 5.54  | 104.67 $\pm$ 22.83 | 77.17 $\pm$ 6.62  |

**Table 4** Anti-hyperglycemic effects of ethanolic extract of leaves of *Leptadenia reticulata* Wight & Arn. 300 mg/kg bw in adrenaline-induced hyperglycemic rats (group II)

| Rat code | Blood glucose concentration (mg/dL) |     |     |     |     |
|----------|-------------------------------------|-----|-----|-----|-----|
|          | Fasting                             | 1hr | 2hr | 3hr | 4hr |
| 1        | 100                                 | 221 | 181 | 101 | 70  |
| 2        | 106                                 | 246 | 206 | 122 | 83  |
| 3        | 93                                  | 254 | 208 | 136 | 86  |
| 4        | 106                                 | 245 | 160 | 108 | 78  |
| 5        | 110                                 | 238 | 102 | 94  | 72  |
| 6        | 114                                 | 256 | 174 | 83  | 68  |

|               |                    |                    |                    |                    |                  |
|---------------|--------------------|--------------------|--------------------|--------------------|------------------|
| Sum           | 629                | 1460               | 1031               | 644                | 457              |
| Mean $\pm$ SD | 126.17 $\pm$ 11.34 | 243.33 $\pm$ 12.74 | 171.83 $\pm$ 38.94 | 107.33 $\pm$ 19.22 | 76.17 $\pm$ 7.33 |
| Mean $\pm$ SE | 126.17 $\pm$ 4.63  | 243.33 $\pm$ 5.20  | 171.83 $\pm$ 15.89 | 107.33 $\pm$ 7.85  | 76.17 $\pm$ 2.99 |

**Table 5** Anti-hyperglycemic effects of 70% ethanolic extract of leaves of *Leptadenia reticulata* Wight & Arn. 600 mg/kg bw in adrenaline-induced hyperglycemic rats (group III)

| Rat code      | Blood glucose concentration (mg/dL) |                    |                 |                    |                  |
|---------------|-------------------------------------|--------------------|-----------------|--------------------|------------------|
|               | Fasting                             | 1hr                | 2hr             | 3hr                | 4hr              |
| 1             | 98                                  | 262                | 190             | 89                 | 76               |
| 2             | 108                                 | 215                | 172             | 125                | 75               |
| 3             | 104                                 | 224                | 201             | 118                | 71               |
| 4             | 108                                 | 239                | 168             | 97                 | 69               |
| 5             | 111                                 | 226                | 184             | 108                | 76               |
| 6             | 98                                  | 235                | 165             | 89                 | 61               |
| Sum           | 627                                 | 1401               | 1080            | 626                | 428              |
| Mean $\pm$ SD | 121 $\pm$ 10.43                     | 233.50 $\pm$ 16.33 | 152 $\pm$ 14.07 | 104.33 $\pm$ 15.17 | 71.33 $\pm$ 5.82 |
| Mean $\pm$ SE | 121 $\pm$ 4.26                      | 233.50 $\pm$ 6.67  | 152 $\pm$ 5.74  | 104.33 $\pm$ 6.19  | 71.33 $\pm$ 2.38 |

**Table 6** Comparison of blood glucose concentrations of normal saline group, positive control group (glibenclamide), ethanolic extract 150 mg/kg, ethanolic extract 300 mg/kg and ethanolic extract 600 mg/kg (Mean  $\pm$  SD, n=6)

| Tested groups                    | Mean blood glucose concentration (mg/dL) |                    |                    |                    |                    |
|----------------------------------|------------------------------------------|--------------------|--------------------|--------------------|--------------------|
|                                  | Fasting                                  | 1 hour             | 2 hour             | 3 hour             | 4 hour             |
| Normal saline group              | 117.17 $\pm$ 20.38                       | 316 $\pm$ 77.24    | 232.33 $\pm$ 48.36 | 133.33 $\pm$ 21.31 | 106.83 $\pm$ 26.19 |
| Positive control (glibenclamide) | 115.67 $\pm$ 18.25                       | 224.50 $\pm$ 27.71 | 187.00 $\pm$ 18.21 | 92.17 $\pm$ 31.14  | 67.83 $\pm$ 17.16  |
| Ethanolic extract 150 mg/kg bw   | 133 $\pm$ 12.51                          | 247.33 $\pm$ 15.36 | 187.83 $\pm$ 13.57 | 104.67 $\pm$ 22.83 | 77.17 $\pm$ 16.22  |
| Ethanolic extract 300 mg/kg bw   | 126.17 $\pm$ 11.34                       | 243.33 $\pm$ 12.74 | 171.83 $\pm$ 38.94 | 107.33 $\pm$ 19.22 | 76.17 $\pm$ 7.33   |
| Ethanolic extract 600 mg/kg bw   | 121 $\pm$ 10.43                          | 233.50 $\pm$ 16.33 | 152 $\pm$ 14.07    | 104.33 $\pm$ 15.17 | 71.33 $\pm$ 5.82   |
| <i>p</i> value                   | 0.29                                     | 0.003              | 0.018              | 0.048              | 0.003              |

#### 4. Discussion

*Leptadenia reticulata* Wight & Arn., commonly known as Goan-kha, is widely distributed in central Myanmar. Although several international studies have reported the anti-diabetic activity of *Leptadenia reticulata* leaf extract, the composition and potency of its bioactive compounds may vary due to several factors, including geographical location, climatic conditions, cultivation practices, and harvest time.

Among the three extracts of *Goan-kha* leaves (petroleum ether, ethyl acetate, and ethanol), the ethanolic extract has been reported to contain flavonoids and exhibit the most potent anti-hyperglycemic effect in streptozotocin-induced rats, according to a previous study[9]. Therefore, the present study utilized 70% ethanol to extract the active compounds from the leaves of *Goan-kha*. The yield of the ethanolic extract was 9.6% following the extraction process.

*Goan-kha* contains a variety of phytochemical constituents across different parts of the plant. A previous study identified 43 chemical compounds in various parts of *Leptadenia reticulata* Wight & Arn.[10]. In the present study, a qualitative analysis was conducted on ten specific phytochemical compounds. The results indicated that the 70% ethanolic extract

of *Goan-kha* leaves contains flavonoids, alkaloids, saponins, tannins, phenols, carbohydrates, amino acids, and resins. However, glycosides and steroids were not detected.

The acute toxicity study was conducted using the OECD 425 method, as it requires a minimal number of animals[13]. A previous study assessing the acute toxicity of *Goan-kha* reported no serious toxic signs or lethality at doses of 1000 mg/kg bw and 2000 mg/kg bw, using the OECD 423 method[9]. In the present study, no lethal effects were observed at doses of 550 mg/kg bw and 2000 mg/kg bw, indicating that the extract is free from acute toxicity or harmful effects. A dose of 5000 mg/kg bw was not tested due to animal welfare concerns, and such a dose should only be administered if the test is directly relevant to protecting human or animal health[13].

In hyperglycemia induction models, alloxan and streptozotocin injections are commonly used to induce permanent pancreatic cell damage and cause diabetes. However, in the present study, adrenaline injection was employed to induce transient hyperglycemia in rats, as the aim was to investigate the anti-hyperglycemic activity under stress-induced hyperglycemia conditions. Intraperitoneal injection of adrenaline in mice has been shown to cause a transient increase in blood glucose and a decrease in liver glycogen content one hour after administration[14]. In albino rats, significant hyperglycemia was observed following intraperitoneal injection of adrenaline at a dose of 0.1 mg/kg bw, which produced a peak hyperglycemic effect at one hour, lasting up to four hours. A previous study used a subcutaneous dose of 0.6 mg/kg bw of adrenaline to induce hyperglycemia in rats, where blood glucose levels rapidly decreased after four hours[14].

In this study, however, the blood glucose level did not increase with a dose of 0.6 mg/kg bw, prompting an increase to 0.8 mg/kg bw, which successfully induced a significant rise in blood glucose levels. Adrenaline was administered subcutaneously, as the intraperitoneal route caused a rapid increase in blood glucose levels, which would not allow adequate time to observe the hypoglycemic effects of the test agent. In a previous study, blood glucose levels peaked at two hours after adrenaline injection and gradually declined after four hours[15]. In contrast, in this study, blood glucose concentrations peaked at one hour and decreased sharply after three hours.

In the present study, the fasting blood glucose levels of all groups ranged from  $115.67 \pm 7.45$  mg/dL to  $133 \pm 5.11$  mg/dL. A previous study by Soe-Moe-Aung[15] reported fasting blood glucose levels ranging from  $94.6 \pm 5.1$  mg/dL to  $110.63 \pm 4.6$  mg/dL. Thus, the fasting blood glucose levels observed in this study were comparable to those reported in the previous study.

In this study, the blood glucose levels in the normal saline group were significantly increased at 1 hour and 2 hours after adrenaline injection. The levels gradually decreased starting at 3 hours. Therefore, adrenaline-induced hyperglycemia was observed at 1 hour, 2 hours, and 3 hours, with the peak occurring at 1 hour. In contrast, the previous study[15] reported that adrenaline-induced hyperglycemia peaked at 2 hours, with significant increases observed at 1 hour, 2 hours, and 3 hours.

Glibenclamide is a commonly used anti-diabetic drug that stimulates the release of insulin from the pancreas to lower blood glucose levels. Its onset of action is typically observed within 2-4 hours, and its duration of action lasts 12-24 hours[16]. This drug is known for its effectiveness in treating diabetes. In the present study, glibenclamide significantly reduced blood glucose levels in adrenaline-induced hyperglycemic rats. A significant decrease in blood glucose was observed at 1 hour and 2 hours ( $p < 0.05$ ). The lower blood glucose levels observed at 3 hours and 4 hours may be attributed to the effects of the tested drug or the extended fasting period.

The lower blood glucose levels were observed at 1 hour and 2 hours ( $p < 0.005$  and  $p < 0.05$ , respectively) following adrenaline injection in rats treated with three different doses of the extract (150 mg/kg, 300 mg/kg, and 600 mg/kg). The reduced blood glucose levels at 3 hours and 4 hours may be attributed to the effects of the tested extract or the prolonged fasting period. Therefore, the anti-hyperglycemic effect was observed starting at 1 hour post-adrenaline injection. In a previous study, the anti-hyperglycemic effect was reported to begin 90 minutes after the administration of an extract at a dose of 200 mg/kg[9]. This study differs from the previous one as it was conducted using adrenaline-induced hyperglycemia, whereas the prior study utilized streptozotocin-induced diabetic rats.

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## 5. Conclusion

This study demonstrated that the leaves of *Goan-kha* possess anti-hyperglycemic effects. Consequently, this plant shows potential for controlling glucose intolerance in individuals with type 2 diabetes and pre-diabetes, particularly under stressful conditions. However, further research, including subacute and chronic toxicity studies, is necessary to evaluate

the long-term safety of Goan-kha. Additionally, investigations into the effect of Goan-kha on insulin sensitivity should be conducted to elucidate the underlying mechanisms of action.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors have no conflict of interest to declare.

### *Statement of ethical approval*

The study was approved by the Academic Board of Post-graduate Studies (Pharmacology) of Defence Services Medical Academy, Yangon, Myanmar. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee.

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