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Original Article

Pasuchaca (*Geranium ruizii* Hieron.): A Medicinal Plant of the *Geraniaceae* Family with Hypoglycemic Effect on Alloxan-Induced Hyperglycemia in Mice

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ABSTRACT

Objective: The current study aims to evaluate the hypoglycemic effect of the hydroalcoholic extract of *Geranium ruizii* on alloxan-induced hyperglycemia in albino mice. **Material and Methods:** *Geranium ruizii* was collected in Huancayo, Junin, Peru. A phytochemical analysis was carried out to confirm the chemical groups. In the pharmacological study, hyperglycemia was induced with alloxan at doses of 170 mg/kg in male albino mice, animals with blood sugar levels above 250 mg/dL were included in the protocol. Mice were randomized into five groups (I: Alloxan 170 mg/Kg; II, III and IV (*Geranium ruizii* extract: 50; 150 and 300 mg/Kg body weight); and V: glibenclamide, 5mg/Kg B.W. **Results:** Phytochemical analysis confirmed the presence of tannins, flavonoids, alkaloids, terpenes, saponins and phenolic compounds. It is observed that at doses of 50, 150 and 300 mg/kg reduced blood glucose at 14 days of treatment. **Conclusion:** The hypoglycemic effect of the hydroalcoholic extract of *Geranium ruizii*, administered orally in mice with alloxan-induced hyperglycemia was effective with 150 mg/Kg body weight.

Key words: *Geranium ruizii*, Antidiabetic drugs, Pasuchaca, Antioxidants, Medicinal plants, Herbal therapy.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases, which is mainly characterized by an elevated blood glucose value (hyperglycemia) and deficient in carbohydrate, fat and protein metabolism.[1] Disturbances in glucose metabolism cause hyperglycemia which plays an important role in the clinical manifestation regarding its side effects and genesis of other concomitant diseases.[2] Around the world is difficult to have a statistical data of diabetes patients, although the World Organization of Health prognostics high rates in the following ten years. For instance, DM type 2 incidence rates are increasing rapidly and the global incidence is projected to arise from 366 to 552 million people by 2030.[3] Currently, the pharmaceutical market sells billions of dollars in medicines for diabetes care, these include metformin, glibenclamide, insulin, among many others.

On the other hand, various studies in plants were developed using animal models for inducing DM chemical reagents such as alloxan and streptozotocin, which are used as inducers of β cell destruction with various mechanisms involving increasing the blood sugar levels after a single administration intraperitoneally in physiological conditions. Although these two experimental models are described widely in the scientific literature, they only represent DM type 1 models due to its mechanism that affects mainly pancreatic cells by oxidative damage or DNA alkylation into the β cells.[4]

Hereby, medicinal plants have also been a good source of natural medicine, specially for in developing countries where synthetic medicine is expensive and with difficult access, increasing the morbidity and mortality rates.[5] Thereby, natural products of medicinal plants could be the best option for reducing the complications generated by DM as well as its administration together with classical drugs for diabetic people, but additional studies have to be carried out to avoid side effect or possible interactions between plants and commercial drugs.

Peruvian flora possesses numerous medicinal plants to treat alternatively DM, for instance, species such as *Geranium dielsianum*, *Geranium ayavacense*, *Gentianella alborosea* and *Annona muricata*. Infusions and teas of these plants are commonly used as a traditional treatment in the peruvian community.[6]

Geranium ruizii Hieron. (Family: *Geraniaceae*) is called "pasuchaca" by peruvian quechua speakers of Cajamarca and Junin. Moreover, this species has a synonym known as *Geranium dielsanium*. Furthermore, species from the Geraniaceae family have hypoglycemic effects and other biological properties such as: antioxidant, anti-inflammatory, analgesic and antimicrobial which it might attenuate the symptoms and serious complications produced by DM.[7] Searching for natural alternatives, our main objective was to determine the hypoglycemic effect of the hydroalcoholic extract of *Geranium ruizii* Hieron. on alloxan-induced hyperglycemia in albino mice.

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MATERIALS AND METHODS

Plant material

3kg of *Geranium ruizii* (whole plant) were obtained from Huancayo, Junin, Peru, in March 2019 at 3.259 meters above sea level. The authentication was carried out in the Herbarium of the Universidad Nacional Mayor de San Marcos (Av. Arenales 1256, Jesús María, Lima 14, Peru). A voucher specimen with 01-USM-2015 number confirmed the scientific name: *Geranium ruizii* Hieron, hereinafter *G. ruizii*, according to our previous studies.[8] A sample of the vegetable species is shown in figure 1. Additionally, Biologist José Campos De la Cruz certified our botanical species.

Extract obtention

Material plant was dried in an oven at 40ºC and pulverized to get a fine powder with subsequently being macerated with 1000 g of *G. ruizii* with 2L of 70 % ethanol for 7 days at 25 ±2°C. Next, the liquid extract was evaporated to dryness using a rotavap. The yield percentage of the extract was 10%.

Phytochemical screening of the hydroalcoholic extract

To carry out the phytochemical analysis, 10 mL of the ethanolic extract of *Geranium ruizii* Hieron was used and diluted with 96% ethanol. The coloration and precipitation reactions were confirmed to determine the presence of characteristic functional groups.[9]

Shinoda reaction: to recognize the presence of flavonoids in a plant extract. An aliquot of the extract were mixed with 1 mL of concentrated hydrochloric acid and a piece of metallic magnesium. A red color confirms the presence of flavonoids.

Gelatin Reaction: 0.5 mL of extract was taken and 0.5 mL of 0.5% aqueous gelatin solution was added. For the determination of tannins, the reaction is positive if turbidity or precipitate appears.

Reaction with Ferric Chloride ($FeCl_3$): 0.5 mL of extract was taken and 1-2 drops of 0.5% aqueous ferric chloride solution were added. For the determination of free phenolic groups, the reaction is positive if an intense blue, black or green color appears.

Figure 1: Photography of different organs of *Geranium ruizii* Hieron.

Reaction for alkaloids: 2 mL of the sample was placed in a test tube and brought to dryness, the residue was dissolved with 5 mL 1% HCl, heating slightly to 50°C, on this solution the Mayer, Wagner and Dragendorff reactions are carried out.

For the tests, 0.5 mL of the solution was taken and drops of the corresponding reagent were added.

Mayer reaction: The reaction is positive if a white precipitate appears.

Dragendorff reaction: The reaction is positive if an orange precipitate appears.

Wagner reaction: The reaction is positive if a brown or dark brown precipitate appears.

Lieberman – Burchard reaction: 1 mL of the sample was taken, 1 mL of acetic anhydrous, and 2 drops of concentrated sulfuric acid were added. For the determination of triterpenoids and / or steroids, the reaction is positive if a blue, green, or orange color appears.

Foam test: An aqueous solution of the sample was subjected to vigorous shaking for 30 seconds. Foaming will confirm the presence of saponins.[10,11]

Experimental animal

30 male Balb/C albino mice (9-10 weeks old) were purchased from the Bioterio of the Universidad Nacional Agraria la Molina-UNALM (District La Molina, Lima, Peru). Mice were fed with a pelletized standard diet (Ratonin ®) with free access to food and water. Mice were divided into five groups and given an acclimatization period of 7 days under standard environmental conditions of temperature (20 ± 2°C), relative humidity (60-70%) and 12 h dark/light cycle, respectively. The present research was approved by the Ethics Committee of the School of Medicine, Universidad Nacional Mayor de San Marcos, Peru, furthermore, Guide for the Care and Use of Laboratory Animals was consulted.[12]

Hypoglycemic effect of the hydroalcoholic extract of *G. ruizii*.

Induction of hyperglycemia in Balb/C albino mice

Hyperglycemia was induced by a single intraperitoneal dose of alloxan monohydrate (Sigma-Aldrich Co) at 170 mg/Kg of body weight, previously solubilized with saline solution (154 mM NaCl) before the induction process. The induction time was controlled between 24 and 48 h after alloxan administration; mice with elevated plasma glucose levels above 250 mg/dL were included in the evaluation of the hypoglycemic effect.[10]

Experimental Design. Six male mice in each group received: Group I: Alloxan (A), 120 mg/kg, intraperitoneal injection. Group II: A + *G. ruizii* (50 mg/kg, per oral), Group III: A + *G. ruizii* (150 mg/kg, per oral), Group IV: A + *G. ruizii* (300 mg/kg, per oral), Group V: A + Glibenclamide (5mg/kg, per oral).

Determination of body weight and blood glucose

Blood samples were taken from the tail tip every seven days, and weights were monitored daily to control the increasing of body weight due to high blood sugar levels. An electronic glucometer with glucose test strips was used to estimate blood glucose levels.

Plasma malondialdehyde level (MDA) assay

The oxidative stress marker was determined in serum from blood taken at 14 days. The thiobarbituric acid reactive species test (TBARS) was used, which generally measures the formation of malondialdehyde and other carbonyls from the oxidative conversion of lipids and other molecules such as proteins. The results will be reported as the formation of the malondialdehyde complex, thiobarbituric acid (MDA-TBA),

a colored complex that is measured at 535 nm. The MDATBA molar extinction coefficient will be used: 1.56 X 10^5 $M^{-1}cm^{-1}$.[13]

Statistical analysis

Data were expressed as the average standard error of the mean of body weight and fasting blood sugar as well as percentage of the hypoglycemic effect and mean of plasma MDA. One-way and two-way analysis of variance (ANOVA) and Tukey and Dunnet poshoc were carried out using GraphPad Prism v6 program. P values less than 0.05 were considered statistically significant.

RESULTS

Phytochemical study of the hydroalcoholic extract of *G. ruizii* Hieron

Following the described methodology, the chemical reactions for each specific metabolite showed the presence of alkaloids, saponins, flavonoids and tannins and high content of phenolic compounds (Table 1).

Body weight of rats treated with *G. ruizii* Hieron

At the beginning, mice weighed ranged 20-22 g. From 1 to 14 days after treatment with *G. ruizii* at various doses, the mean body weight in both treated and control groups increased steadily at 7 days. However, in the experimental groups, there was a trend for the gain in mean body weight to slow down after about 7 days (Figure 2). There was a statistically significant difference between the body weights of alloxan group and *G. ruizii* group at 150 mg/kg at 14 days of treatment.

Hypoglycemic effect of hydroalcoholic extract of *G. ruizii* Hieron

The hydroalcoholic extract of *G. ruizii* was orally administered to alloxan induced hyperglycemia mice at single doses of 50, 150 and 300 mg/kg for 14 days. Blood glucose level was significantly decreased in mice treated at a dose of 50 mg/Kg ($P < 0.0001$), 150 mg/Kg ($P < 0.0001$) and 300 mg/Kg ($p < 0.0001$) in 0.5 hours after treatment relative to the alloxan group, which only received distilled water at 10 mL/Kg (Figure 3). A sharp decline in blood glucose level at the dose of 150 mg/kg occurred at 24h and 7 days, which there was not a significant difference with the glibenclamide group. However, all treatment with extracts and glibenclamide had hypoglycemic effect during the 14 days of evaluation.

Table 1: Phytochemical analysis of the ethanolic extract of *G. ruizii*. Hieron.

Metabolite Secondary	Test	Results
Tannins	Gelatin reaction	+
Phenolic compounds	Ferric Chloride ($FeCl_3$)	+++
Alkaloids	Dragendorff reaction	+
Alkaloids	Wagner reaction	+
Alkaloids	Mayer reaction	+
Flavonoids	Shinoda reaction	+
Triterpene / steroids	Lieberman Burchard reaction	++
Saponins	Foam test	+

Legend: Absence (-), Mild Presence (+), Moderate (++), Intense (+++)

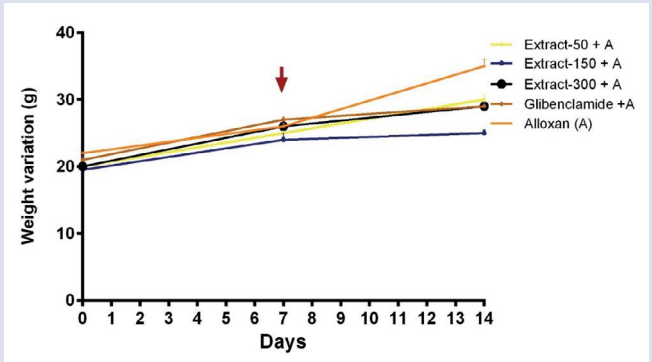

Figure 2: Variation of body weight between *G. ruizii* group and alloxan group from 1 to 14 days of evaluation.

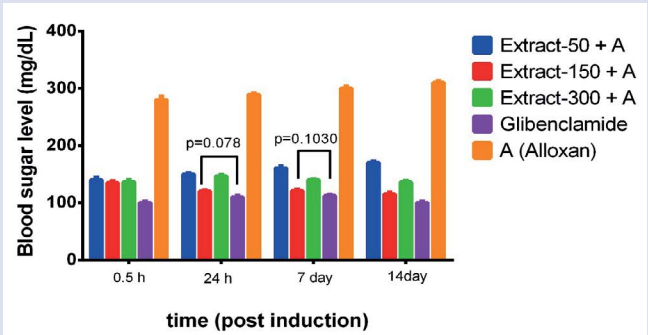

Figure 3: Blood sugar levels in mice treated with *G. ruizii*. ANOVA, $P < 0.0001$; Dunnet Test: *P < 0.05 were considered significant between groups and mice treated with glibenclamide.

Regarding blood sugar variation expressed in percentages, at 0.5h after the extracts and glibenclamide administration, better results were showed in those mice treated with the gold standard (39.5%) followed by *G. ruizii* at 150 mg/Kg (25.6%) and 300 mg/Kg (30.5%) respectively. However, glibenclamide and *G. ruizii* at 150 mg/Kg presented 70.7% and 66.5%, respectively, at 14 days (figure 4).

Plasma MDA level assay

MDA is a biochemical antioxidant marker, which was measured in mice at the end of the experimental study. Mice treated with 50 and 150 mg/kg of *G. ruizii* had better reduction values compared to mice receiving a doses of 300 mg/kg, additionally, all treatments with 50 mg/kg, 150 mg/kg and glibenclamide group had significant differences compared to the alloxan group (Figure 5).

DISCUSSION

Experimental hyperglycemia was induced using alloxan as a chemical toxic, which produces a selective toxicity on the selective cells of the pancreas and when causing damage, it produces a decrease in the level of insulin and induces a state of type I diabetes mellitus. The mechanism of action of alloxan is produced by its similarity with the chemical structure of glucose, in this way alloxan is retained by the beta cell of the pancreas *via* $GLUT_2$ transporter, with low affinity to the plasma membrane. alloxan can produce reactive oxygenated species (ROS) in a cyclical redox reaction, producing dialuric acid (AH_2), in this way alloxan produces free radicals intracellularly in the pancreatic beta cell.[14] The autoxidation of dialuric acid (AH_2) generates superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2) and finally hydroxyl radical (OH^-). The autoxidation of radical AH_2 implies the intermediate formation of the radical alloxan (AH_2).[10] The reduction of alloxan to AH_2 in the pancreatic beta cell requires thiol groups such as glutathione tripeptide (GSH) and other intracellular thiols present in minimal concentrations such as cysteine monothiol and dithiols. Therefore, the ability of cells to capture alloxan *via* GLUT-2, which decreases the antioxidant defense potential against oxidative stress and the formation of hydroxyl radicals, explains the selective toxic action of the beta cells of the pancreas as well as the hyperglycemic effect of alloxan.[15]

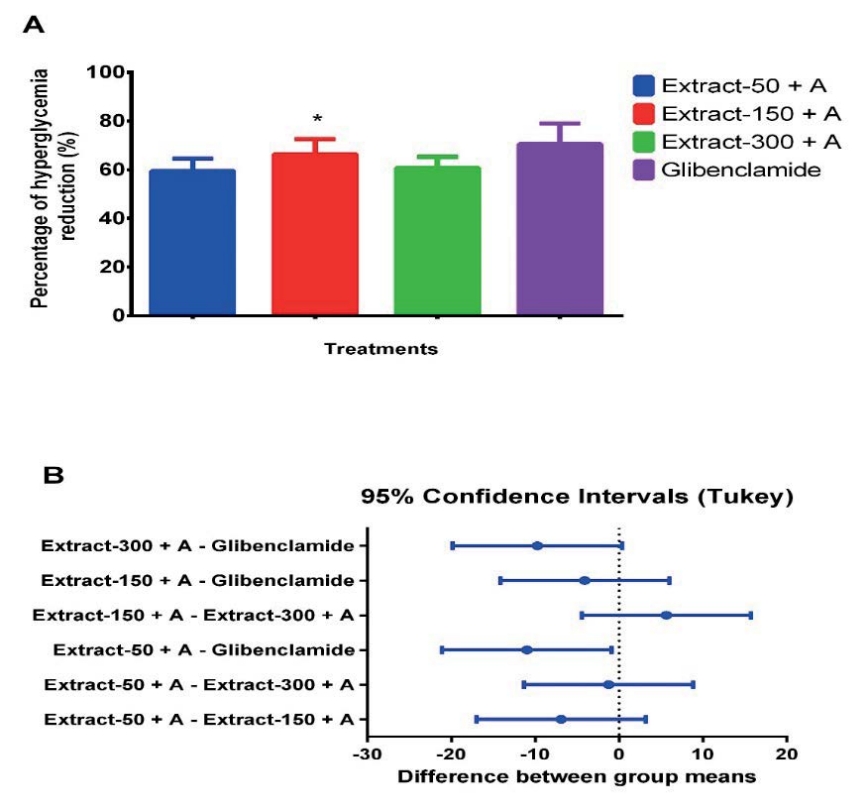

Figure 4: A. Percentage of reduction in blood sugar level in mice treated with *G. ruizii* for 14 days. ANOVA, *P* = 0.0216; Tukey Test: *P < 0.05 between the extract-50 group and glibenclamide group. B. Difference between group means at 14 days after hyperglycemia induction.

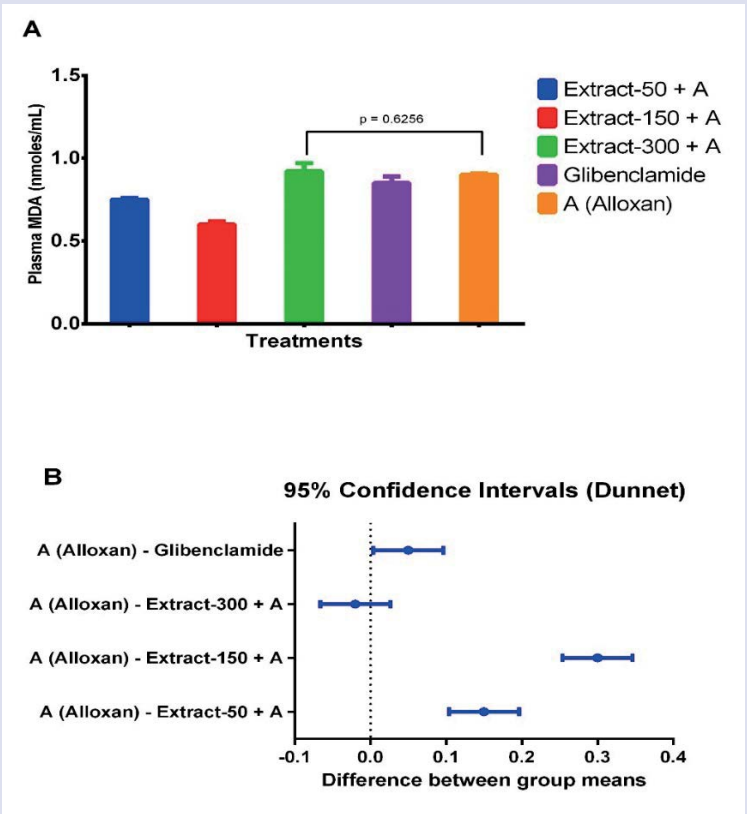

Figure 5: A. Plasma MDA in mice treated with *G. ruizii* for 14 days. ANOVA, *P* <0.0001; Dunnet Test: *P < 0.001 between group, experimental groups and alloxan group. B. MDA value difference between group means at 14 days after hyperglycemia induction.

Alloxan can produce reactive oxygenated species (ROS) in a cyclical redox reaction, producing dialuric acid (AH_2), in this way alloxan produces free radicals intracellularly in the pancreatic beta cell. The autoxidation of dialuric acid (AH_2) generates superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2) and finally hydroxyl radical (^{-}OH).[16] Therefore, the ability of cells to capture alloxan *via* GLUT-2, which decreases the antioxidant defense potential against oxidative stress and the formation of hydroxyl radicals, explains the selective toxic action of the beta cells of the pancreas as well as the hyperglycemic effect of alloxan.[17] Given the presence of phenolic compounds present in the extract of pasuchaca, especially the flavonoids that have different activities such as hypoglycemic. Quercetin activates hexokinase and glucokinase inhibiting α, β glycogen phosphorylase and glucose-6-phosphatase in liver.[15] Considering that obesity linked to type II diabetes is associated with a low degree of inflammation, it has been proposed that polyphenols can protect against the damage caused by type II diabetes through their anti-inflammatory effect. Currently, polyphenols such as curcumin, capsaicin, gingerol, catechin, resveratrol and quercetin have demonstrated their anti-inflammatory effects by directly blocking the mitogen-activated protein kinase (MAPK) pathway, NFkB activity and the expression of anti-inflammatory cytokines.[8] Although, we did not analyze the phytochemical structures, the extract might work inhibiting maltase or α-glucosidase activity, according to a report on this plant.[18]

CONCLUSION

As a conclusion, we demonstrated that *G. ruzii* at doses of 150 mg/kg reduced blood sugar levels at 14 days of treatment and could be considered a good alternative to the management of diabetes.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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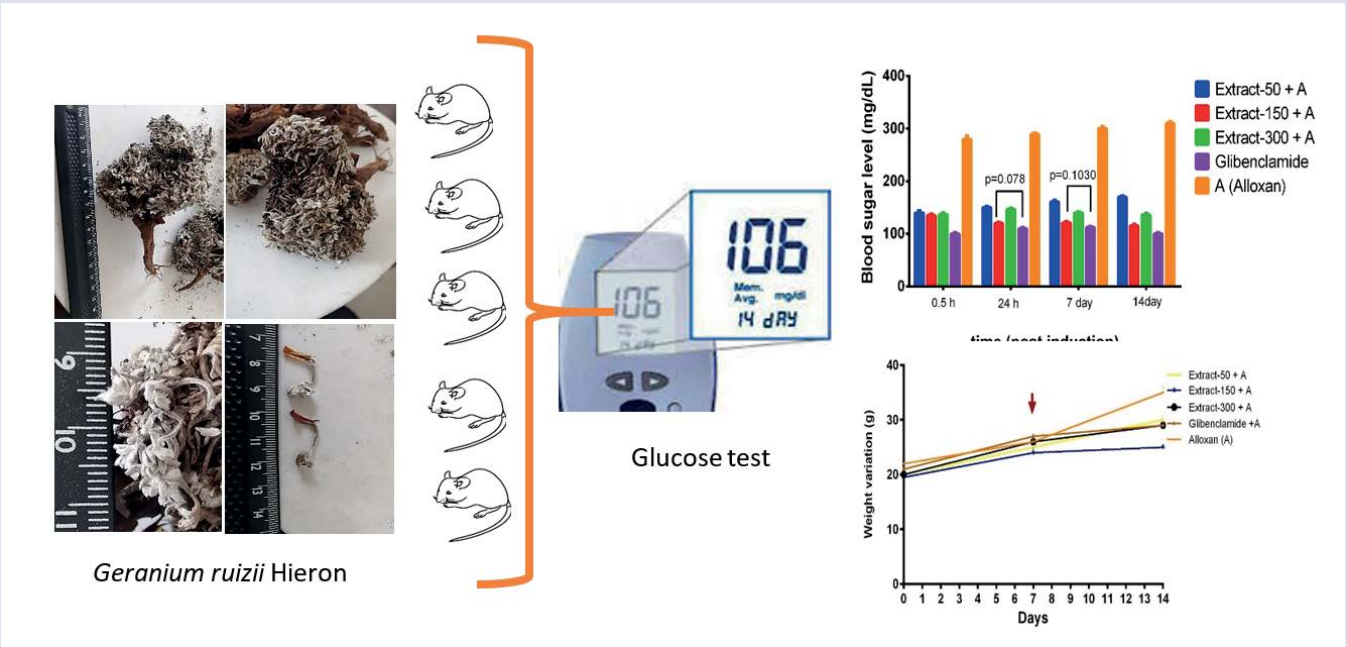
GRAPHICAL ABSTRACT



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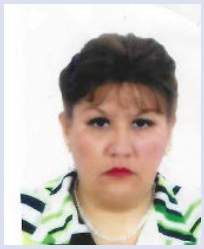

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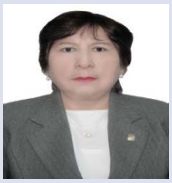

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