Research Article

Hypoglycemic Effect of *Moringa oliefera* Lam. Seed Oil Extract and Royal Jelly Composite Mixture in Alloxan-Induced Diabetes in Male Albino Wistar Rats

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Submission Date: 13-03-2021; Revision Date: 24-04-2021; Accepted Date: 25-04-2021

ABSTRACT

Artificial diabetes was introduced to healthy male albino wistar rats to establish the hypoglycemic effect of the combined *Moringa oleifera* Lam. seed oil (MOSO) extract with royal jelly (RJ). *Moringa oleifera* Lam. seed oil was afforded via Soxhlet extraction with hexane and subsequent phytochemical analysis was performed revealing the presence of alkaloids and flavonoids. There were five treatment groups (pure MOSO, MOSO-10, MOSO-15, MOSO-20, and pure RJ) and a negative control group used in this study. The treatment with 20% loading (MOSO-20, Treatment D) of the seed oil extract in royal jelly was found to be the fastest to normalize the blood glucose levels in seven days, followed by Treatment E, Treatment C, Treatment B, and Treatment A. Analysis of variance and Tukey's HSD *post hoc* analysis of data revealed that treatment groups had results which were significantly different ($p < 0.05$). Results of this research can be used to explore the synergistic effects of MOSO and RJ in the future.

Key words: Antihyperglycemic, Blood glucose, *Moringa oleifera,* Royal jelly.

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INTRODUCTION

Prevalence of patient with diabetes mellitus worldwide has increased accordingly by year.[1] According to the Diabetes Atlas, 382 million people worldwide have DM in 2013 and are expected to reach up to 592 million in 2035, with 80% of them living in low to middle income countries.[2] Based on the last survey of the world population of DM, Asian countries are still on top comprising the 60% of the total population of DM.[3] Among these Asian countries with DM, China has the greatest number of cases of DM with a population of 98.4 million, followed by India with 65.1 million cases.[4] In 2017, according to International Diabetes federation, there are almost 3,721,900 cases of DM and gradually increase by year in the Philippines. By 2030, it is expected that DM will become the seventh leading cause of death in the world, making it a growing health concern in our society.[5]

High medical costs of diabetes treatment plagued patients for the longest time and so research on herbal medicines that may control blood glucose levels with lower the risk of complications is essential.

Moringa oleifera, known as miracle tree for the many purposes of its every part, has shown potential in leading to reduce blood sugar levels. With its high nutritive values, every part of the tree is suitable for either nutritional or commercial purposes.[6]

Royal jelly on the other hand is a milky secretion produced by worker honeybees. It typically contains about 50% to 70% water, 9% to 18% proteins, 7% to 18% carbohydrates, 3% to 8% lipids, and 2% to 3% polyphenols, enzymes, hormones, vitamins, salts, and amino acids. Its composition varies depending on geography and climate.[7]

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DOI: 10.5530/ajbls.2021.10.23

Although there have been numerous reports on the anti-hyperglycemic effects of *Moringa oleifera* extracts[8-10] there have been only handful of studies on its oil as hypoglycemic agent. Hence in this study, we aim to find out if there is hypoglycemic effect of combined *Moringa oleifera* seed oil (MOSO) extract and royal jelly on the blood glucose levels of alloxan-induced diabetes in albino wistar rats.

MATERIALS AND METHODS

Plant Material

Healthy and young *Moringa oleifera* Lam. pods were gathered after purchasing it in the market around Quiapo, Manila, Philippines. It was authenticated by a botanist at the National Museum of the Philippines Botany Division located at Padre Burgos Ave, Ermita, Manila, Philippines. A voucher specimen was stored in herbarium for reference purposes.

Preparation and Oil Extraction of Moringa oleifera Lam. seed

The pods containing the seeds were air-dried at average room temperature and was kept away from high temperatures and direct sunlight to avoid the degradation of active compounds. After two weeks, the pods were opened, exposing the seeds. The seeds were ground to fine powder using mortar and pestle. All products were weighed using an analytical balance.

The pulverized seeds were subjected to Soxhlet extraction with *n*-hexane as the solvent according to the methods of Alhassan[11] and Akinyeye[12] published elsewhere. The process was repeated automatically until complete extraction was obtained. The *Moringa oleifera* seed oil (MOSO) extracts were stored at 4°C in amber airtight bottles.

Phytochemical Screening Test

Phytochemical screening of the MOSO extract was performed for qualitative detection of alkaloids, flavonoids, reducing sugar, tannins, and saponins. The tests were performed based on the methods of described elsewhere.[9]

Royal jelly

Freshly frozen royal jelly (RJ) was procured from *Ilog Maria* Honeybee Farm in Silang, Cavite. The royal jelly was stored at sub-zero temperature of -4°C until used for mixing with the MOSO extract and for the treatment. To reduce the viscosity of royal jelly, normal saline solution (NSS) was mixed with it in a 1:1 ratio.

Preparation of Treatments

Three different concentration of treatments were prepared from mixing MOSO in RJ such as 10% (B), 15% (C), and 20% (D). The two component substances were firstly mixed with a magnetic stirrer and then after through vortex mixing. Meanwhile, Treatment A was pure MOSO, and Treatment E was pure RJ. In the following sections, these treatments were given to Albino Wistar rats via intraperitoneal injection.

Albino Wistar Rats as Test Subjects

A total of 36 healthy male albino Wistar rats with body masses ranging from 150-200 g were used in this experimental study. They were procured at Pet Town Pet Supplies and Animal Services, in Quezon City, Philippines. The albino Wistar rats were housed and tested in the Animal Care Use Program (ACUP) at the De La Salle Araneta University in Malabon City, Philippines.

Before inducing diabetes and subsequently administering the treatments, the animal subjects were housed in polypropylene cages under standard environmental conditions: equal amounts of light time and dark time (12 h each) in a temperature range of 25 ± 2°C, humidity of 35-60% with enough air ventilation. Moreover, standard pellet diet and fresh water were given to the rats *ad libitum*. The test animals were randomly divided into six (6) groups with six (6) animals in each group. Group A was treated with MOSO only (Treatment A), Group B was treated with 10% MOSO (Treatment B), Group C was treated with 15% MOSO (Treatment C), Group D was treated with 20% MOSO (Treatment D), Group E was treated with RJ mixed with normal saline solution (NSS, 1:1), and Group F was treated with distilled water as placebo.

Alloxan-Induced Diabetes in Albino Wistar Rats

Induction of diabetes using alloxan monohydrate (Aldrich) in albino Wistar rats was carried out according to the guidelines outlined by Oghenesuvwe.[13] The experimental animals were weighed initially before introduction of artificial diabetes. After 7 days, the animals were grouped into 6 (A-F). The following equations were used to calculate the (a) required dose for a particular weight of rat (RD), (b) equivalent dose in mL (ED), and (c) bulk volume of the stock solution (BV).

Equation a:

$$RD = \frac{\text{wt. in g of rat}}{1000\text{g}} \times \text{Standard dose (mg)}$$

Equation b:

$$ED = \frac{\text{wt. in g of rat}}{1000g} \times \text{volume of vehicle in mL}$$

Equation c:

$$BV = \frac{ED}{RD} \times 300\text{mg of alloxan monohydrate}$$

Using the above-mentioned equation, for test animals with body weight of 150-g, a dose of 22.5 mg is prescribed or an equivalent dose of 0.30 mL. On the other hand, animals weighing 160-g will receive a dose of 24.0 mg or 0.32 mL and so on. In all cases, the standard dose of alloxan monohydrate should be 150mg/kg.

The administration of alloxan was done intraperitoneally using 1-cc syringe. After 72 hr, the rats with sugar level between 200-300 mg/dL or higher were considered experimentally diabetic. For storage of alloxan, it was stored in a vial for further use.

Blood Glucose Determination

The method performed in glucose determination was according to methods described by Ezeigbo with some modifications.[14] The baseline glucose was determined after the rats are acclimated to a new environment. The rats were then abstained from food or drink after injection of alloxan. After 2 to 3 days if the rats are considered diabetic, they were administered with the extracts for 2 weeks. The blood samples were collected from the vein puncture for the determination of the blood glucose level in the fasting animals using a glucometer (GlucoLeader™ Value). To reduce the stress from the test animals, glucose levels were taken after three days, five days, seven days, and 14 days from the moment the animals developed hyperglycemia.

Data Analysis

Statistical Package for the Social Sciences (SPSS) 27.0 was used for the data analysis of this study. The blood glucose level results were expressed as mean of the six rats in each group. All data were analyzed using one-way analysis of variance (ANOVA) as it is used in comparing two or more groups and also assess if there are significant differences among the groups being tested. Tukey's *post hoc* analysis and t-test were also used to further pinpoint the significant results.

Ethical Considerations

Ethical clearance for animal usage in this study was obtained from the Bureau of Animal Industry of the Department of Agriculture with reference number AR-2017-029.

RESULTS

Phytochemical Screening of MOSO

Phytochemical screening of the MOSO extract was performed for qualitative detection of alkaloids, flavonoids, reducing sugar, tannins, and saponins. The tests were performed based on the methods of described elsewhere.[9]

The present study reveals that MOSO extract contains alkaloids and flavonoids. Alkaloids was confirmed by Wagner's reagent with gave reddish brown precipitate upon introduction to the extract. Using 10% sodium hydroxide solutions, intense yellow colored solution was observed upon introduction with MOSO confirmed the presence of flavonoids. However, the yellow tinge disappeared with the addition of dilute hydrochloric acid. Moreover, we were not able to detect the presence of tannins, saponins, and reducing sugars from the MOSO extract (Table 1).

Artificial Hyperglycemia in Rats

Diabetes was artificially induced to test animals using alloxan monohydrate. After 48 hr of introducing the drug intraperitoneally, animals with blood glucose levels higher than 200 mg/dL were considered hyperglycemic. In this study, all test animals had blood glucose levels

Table 1: Summary of Phytochemical Screening of MOSO extract

Phytochemical	Test Reagent	Observed Result	Inference
Alkaloids	Wagner's	Reddish brown precipitate	Positive
Flavonoids	10% NaOH solution	Intense yellow solution that becomes colorless after addition of dilute acid	Positive
Tannins	0.1 M $FeCl_3$ solution	Blue-black coloration	Negative
Saponins	Froth test	No Foam formed	Negative
Reducing Sugars	Benedict's	No brick-red precipitate	Negative

higher than 200 mg/dL (mean = 367 mg/dL). The blood glucose levels of the rats were taken after 3, 5, 7, and 14 days.[14]

Hypoglycemic Effect of MOSO

The hypoglycemic activity of the five treatments namely: pure MOSO, MOSO-10, MOSO-15, MOSO-20, pure RJ and the negative control was tested on alloxan-induced diabetes in male albino wistar rats. The blood glucose levels of the rats were taken after 3, 5, 7, and 14 days.[14]

Based on the Figure 1, it was observed that among the five treatments, the treatment with 20% MOSO loading (Treatment D) started to show significant decrease in blood glucose levels as early as three days (T3) compared to their hyperglycemic glucose levels. In the fifth day (T5) reading, animal subjects in this group are already in borderline diabetic levels (BGL > 200 mg/dL). The glucose levels of this experimental group already had no significant difference compared to their baseline glucose levels at day seven, T7 ($p < 0.05$). It was also observed that the consistency of Treatment D was the best compared to other Treatments used in this study.

Treatment B started to significantly lower the BGL at five days while Treatment C manifested the hypoglycemic effect at three days. Blood glucose levels of the test animals in Groups B and C were already below the diabetic level threshold at days 14 and 7, respectively.

For the pure MOSO (Treatment A) and pure RJ (Treatment E), data reveals that hypoglycemic effects were observed at Day 14 and Day 5, respectively. In both cases, the measurement for these groups at Day 14 suggests that it is already statistically the same with their baseline BGL.

Results reveal that after 14-days, all the treatments significantly lowered the blood glucose levels of the rat samples ($p < 0.05$) compared to their diabetic blood glucose levels. On the one hand, Group F, the negative control showed no improvement.

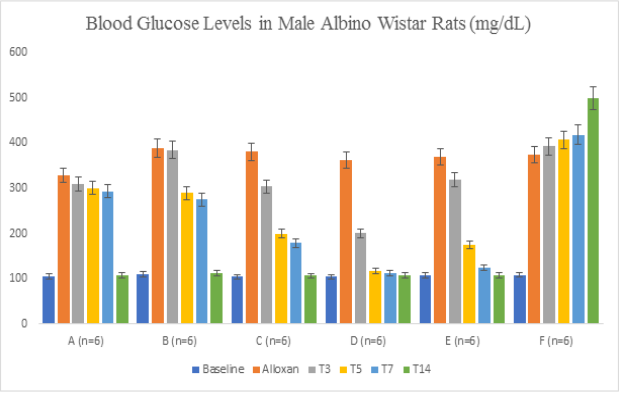

Figure 1: Summary of blood glucose levels of Albino Wistar Rats.

Based on Tukey's *post hoc* analysis of the results, Treatment D (MOSO-20) lowered the elevated blood glucose levels in male albino wistar rats the fastest, opening a potential cure for diabetes type II. The hypoglycemic effect of this treatment normalized blood glucose levels of diabetic rats a week which is twice as fast than the other treatments with lower loading of MOSO, i.e.,10% and 15%.

DISCUSSION

Phytochemical Screening

Upon Soxhlet extraction, the MOSO extract was tested for its phytochemical constituents in which alkaloids and flavonoids were afforded. Alkaloids are naturally occurring amines that have pharmacological effects on humans and animals. Alkaloids have been reported to suppress the transfer of the disaccharide sucrose from the stomach going to the small intestine where it is degraded into glucose molecules.[15]

On the other hand, flavonoids are bioactive secondary plant metabolites which have hypoglycemic as well as antioxidant properties. Generally, flavonoids improve the glucose and oxidative metabolism which is impaired during in hyperglycemic states.[16] It was also reported that flavonoids enhance insulin release and regeneration of pancreatic beta cells.[17] These results suggest that *Moringa oleifera* seed oil extract possesses phytochemicals that aid in lowering blood glucose levels.

Artificial Hyperglycemia in Rats

Alloxan monohydrate was used to introduce artificial diabetes in male Albino Wistar rats. In this present study, the blood glucose levels of the animals spiked (mean = 367 mg/dL). This result implies that the four-phase action of alloxan is complete wherein total degranulation and loss of beta cells integrity is evident while non-beta cells remain intact.[18] For this permanent hyperglycemic phase, alloxan has caused an insulin-dependent type 1-like diabetes syndrome.[19]

Hypoglycemic Effect of MOSO

Treatment D showed statistically significant blood sugar-lowering activity as early as Day 3. It implies that treatment D experimentally cured hyperglycemia in rats for this group. The hypoglycemic effect of this treatment could be attributed to the alkaloids and

flavonoids present in MOSO and high concentration of major royal jelly proteins (MRJP 1-9) and free amino acids in RJ.[20]

Meanwhile, other composite mixtures of MOSO and RJ namely: MOSO-10 and MOSO-15 lowered blood glucose levels but in a slower pace. According to the composition of the mixture, it could be inferred that higher concentration of MOSO is more advisable than opting for lower concentration.

Pure MOSO (Treatment A) and pure RJ (Treatment E) normalized the sugar levels of their respective groups after two weeks. These results are in congruence to report of Busari[21] wherein, *Moringa oleifera* seed oil can serve as a source of potential hypoglycemic agent or adjuvant. In another study, MOSO extract exhibited a hypoglycemic effect on both the mild and severe alloxan induced hyperglycemic rats. Meanwhile, the glucose lowering effect of royal jelly[22] and its use as an effective functional food to prevent insulin resistance associated with the development of hypertension was also reported.[23]

CONCLUSION

The rat-race to find a sustainable and cost-effective cure for diabetes is still on the run and so the potential of *Moringa oleifera* seed oil extract loaded in royal jelly was explored in this study. The hypoglycemic effect of composite mixtures of *Moringa oleifera* seed oil extract and royal jelly was tested on Alloxan-induced diabetes in male Albino Wistar rats. Findings of this research reveal that as the concentration of MOSO is increased, its effectiveness is also follows. The composite treatment D in which the MOSO loading was 20% also proved to be more effective in lowering the sugar levels of the test animals that the pure MOSO or pure royal jelly. The findings of this study also hints of the supra-additive synergistic effects of MOSO and RJ where in the effect of the combined substances is greater than the individual effects of the substances mixed, although more experiments will be needed.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Department of Medical Technology, Far Eastern University – Manila for the usage of the laboratories and reagents in this study.

Author Contribution Details

Macadangdang, RJR conceptualized and designed the experiment, and approved the final version of the manuscript. Orodio, Calabio, Miciano and Tan performed animal handling and laboratory extractions. All authors contributed in the data analysis and interpretation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Cite this article: Macadangdang RJR, Orodio ALT, Calabio AGT, Miciano KBV, Tan ACL. Hypoglycemic Effect of *Moringa oliefera* Lam. Seed Oil Extract and Royal Jelly Composite Mixture in Alloxan-Induced Diabetes in Male Albino Wistar Rats. AJBLS. 2021;10(1):159-64.