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Mollugo oppositifolia leaf tea formulation and its effect against alloxan-induced hyperglycemic male sprague dawley rats

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Abstract

Hyperglycemia is one of the most prevalent health concerns worldwide which is rapidly increasing in middle and low–income families. Though *Mollugo oppositifolia* Linn. Is found throughout the Philippines, its blood glucose-lowering activity in its tea form has not been studied. The study was conducted to determine the hypoglycemic effects in tea – bag and spray – dried form on Sprague Dawley rats injected with 150 mg of alloxan prepared in 0.9 sodium chloride. It is noted that both tea forms have no significant difference as the commercial drug (Glibenclamide) in lowering the blood glucose concentration. This study also evaluated the formulated leaves based on its moisture content, ash content, and microbial determination, which are all within the acceptable range. Sensory evaluation was also done, and it was found that the taste, color, and aroma show characteristics expected of a green tea. It is recommended that a histopathological test should be conducted on the liver. Furthermore, that the bitter after-taste be masked.

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Introduction

Hyperglycemia is common during critical illness and occurs in individuals with and without a previous history of diabetes. The finding supports observational studies demonstrating that elevated blood glucose is associated with morbidity and mortality in hospitalized patients that correction of hyperglycemia can improve outcomes (Falciglia *et al.,* 2009). Impaired fasting hyperglycemia, impaired glucose tolerance, and diabetes mellitus are seen as progressive stages of the same disease process, and treatment at earlier stages has been shown to prevent progression to later stages (by diet, exercise and lifestyle management).

The number of cases for diabetes, which is currently at 171 million, is predicted to reach 366 million by the end of 2030 (WHO, 2017). The global expenditures for diabetes treatment are expected to grow from 760 billion U.S. dollars to 845 billion U.S. dollars from 2019 to 2045 (Eflein, 2019). Numerous studies have proven that plants belonging to the same family as to *M. oppositifolia* like *Mollugo pentaphylla* and *Glinus oppositifolius* possess hypoglycemic effects on alloxan-induced hyperglycemic rat models (Simpson and Morris, 2014). This prostrating annual herb has numerous folkloric uses such as its anti-inflammatory and analgesic activity (Gopinathan, 2014).

It is used in itch and other skin diseases and for suppression of the lochia. Tribes in South India use this plant to cure liver diseases and diabetes. *M. oppositifolia* is commonly found in towns at low and medium altitudes.

It prevalent in India, Philippines, North America, tropical Africa, Australia, and numerous parts of Asia.

As consumers shift their consumption to healthy products, tea is being preferred on account of various health benefits offered by it. Hence, this study aims to formulate spray-dried and tea-bag forms of *M. oppositifolia* as well as to determine its hypoglycemic effect on the blood glucose level of alloxan-induced hyperglycemic Sprague Dawley Rats.

Materials and methods

Phytochemical screening

Preparation of plants for extraction: Plant leaves were brushed briskly to remove visible soil and dust particles by deionized water as quick as possible. Plant parts were placed on a spin drier and blotted off into filter paper, then weigh and put in an oven. Plant parts were oven-dried at 40^{0}C for 12 to 72 hours. Samples were ground finely in a heavy-duty grinder. Samples are kept in sealed polyethylene bags, appropriately labeled, and stored before analysis.

Aqueous extraction: Ten grams of dried material with constant weight was weighed and soaked in 90ml of solvent. It was placed on a hot plate at 50^{o}C for 2 hours with continuous stirring. After 2 hours, it was placed overnight in a refrigerator at 4^{o}C. Then it was placed in a hot plate at 50^{o}C for another 2 hours at constant stirring. The extract was then collected and stored in a refrigerator until further use.

Qualitative Analyses of Secondary Metabolites: Standard protocols were conducted to test for the presence of alkaloids, carotenoids, combined anthraquinones, coumarins, flavonoids, quinones, saponins, steroids, tannins, terpenoids, and xanthoproteins.

Gathering of leaves and tea development

The plant was obtained at Mabbang, Rizal, Philippines with the use of sterile gloves. The fresh leaves of *M. oppositifolia* were washed with distilled water, and the roots were carefully and thoroughly removed.

For the spray-dried tea, the obtained parts were boiled for 5 minutes and were put in a container to be cooled using some ice. After this process, the leaves were cut into smaller pieces using sterile scissors and put into a blender.

The ground leaves were pounded using a pestle, and the extract is obtained by squeezing with the use of sterile gloves and was mixed with 12 liters of distilled water ran through a funnel and strainer.

For the tea bag, the leaves were washed thoroughly through a running water with use of gloves. The leaves are then separated from their roots. Two kilos of obtained leaves are rewashed using distilled water. It is then placed to a primary strainer to dry. The partly dried leaves are placed in the cabinet drier to be dried thoroughly and also to prevent contamination with the air.

Analysis of tea

Determination of moisture Content: Moisture content was determined using a moisture analyzer.

Ash determination: The ash content was determined using the gravimetric method of analysis.

Microbial analysis: Aerobic platecounts were determined using the pour plate method, while total coliform was analyzed using the enzyme-substrate test.

Acute oral toxicity study

Single-dose toxicity study was done according to the Organization for Economic Co-operation and Development (OECD) guidelines no. 423 adopted for acute toxicity in animals up to 2 g/kg (limit test) for the tea bag and 2000 mg/kg for the spray-dried tea. For this purpose, ten rats were used in total in accordance with the guidelines. Both tea forms did not show any signs and features of toxicity or mortality up to 2.0 g/kg per oral dose for the tea bag and 2000 mg/kg per oral dose for spray-dried tea in Sprague Dawley rats.

Animal preparation

Eight to fifteen week–old healthy adult male Sprague Dawley rats were used. They weighed between 150–200g and they were housed at the Philippine Institute of Traditional Alternative Health Care laboratory. The animals are allowed to acclimatize under laboratory conditions 25±20°C following the OECD Guideline 401. They were kept 12:12 light and dark cycle in polypropylene cages for a period of 5 days prior to the experiment. Animals were fed with standard rat chow pellet and provided water ad libitum.

Preparation and Intraperitoneal administration of Alloxan

Alloxan was prepared by adding 700 mg of alloxan to three ml of normal saline. A dose of 150 mg/kg of alloxan was used by the researchers for the induction of diabetes to the rats. Alloxan was administered intraperitoneally to the rats following the International Animal Care and Used Committee (IACUC) Standard Procedure on Animal Induction in Mice 2014.

Induction of hyperglycemia

The prescribed 150 mg/kg of Alloxan was administered intraperitoneally to induce hyperglycemia in Sprague Dawley Rats based at the MSDS of Alloxan. It has been administered after the fasting of the experimental mice and before the administration of the tea. The administration was done intraperitoneally, specifically on the lower left quadrant since, in this area we can inflict the least damage to the organs except in the small intestine.

Tea preparation and administration

The rats were segregated into four groups. The first group treated with *M. oppositifolia* in tea-bag. This was prepared by adding 200 ml of boiling water to 1 tea bag (2 g of tea leaf) and steeping for 10 mins (Hosoda *et al.*, 2003). The second group was treated with the spray-dried powder tea. The tea was prepared using 1500 milligrams of the powder infused into 150 ml of hot water and stirred for 1 minute (Tsuneki *et al.*, 2004).

Treatment groups

The first two groups were the treatment groups; the first group was treated with the tea bag, the second group was treated with spray-dried powdered tea, the third group was the positive control group, treated with Glibenclamide, and the final group was the negative control using distilled water.

Statistical analysis

Paired t-test compared the base to the 12-hour post-treatment blood glucose level and further analyzed using Scheffe post-hoc analysis. Paired t-test between samples was also done to determine if these are comparable. The hypothesis is tested at 0.05 level of significance. All statistical analysis was performed using SPSS version 20 software.

Descriptive procedure

Respondents of the study: Twenty-five respondents who are willing to cooperate in the study were included. The respondents included 14 males and 11 females, age of the participants can be categorized into three age groups: 13 were Adults (30 – 64), 8 were young adults (18-29), and 4 were children (under the age of 18).

Sampling design

The respondents were chosen through convenience sampling. Instrumentation: Respondents were interviewed face-to-face using a modified questionnaire adopted from Ferrara *et al.* (2014) with modifications to adapt to the requirements needed by this study.

Data analysis

The results of the consumer acceptability test were analyzed using mean and using the Likert scale with Hedonic description in the interpretation of the data that resulted in a mean. The mode and frequency were used to analyze the sensory evaluation of the tea.

Results and discussion

Qualitative phytochemical analysis of *M. oppositifolia* aqueous extract revealed the presence of tannins, flavonoids, saponins, triterpenoid acids, and phenolic compounds. Tannins, flavonoids, and phenolic compounds demonstrated anti-diabetic activities (Gopinathan *et al.*, 2014).

Table 1. Physicochemical and microbiological analysis of the tea products.

Sample	Moisture %	Ash %	Aerobic plate count	Total coliform count
Tea – bag	4.30	0.68	$9.4x10^3$ CFU/g	<3.0 MPN/g
Spray – dried powdered tea	4.45	0.68	$9.2x10^3$ CFU/g	<3.0 MPN/g

The role of these plant secondary metabolites is documented in several studies as antidiabetic agents involving different targets in the treatment such as regulating impaired glucose metabolism, β-cell functioning, GLP-1 homeostasis, and restoration of insulin levels (Ahmed, 2012; Arif *et al.*, 2014; Osadebe *et al.*, 2014; Jerine and Sabina, 2014; Sonkamble *et al.*, 2018).

Table 2. Mean concentration of glucose in Sprague-Dawley rats before and 12-hours after the administration of treatments and control.

Treatment	Before administration (mg/dl) (n=5)	12-hours after administration (mg/dl) (n=5)
Tea bag	269.8000	110.4000
Spray-dried tea	309.4000	120.4000
Positive control	323.0000	100.2000
Negative control	259.0000	286.8000

Saponins have many beneficial effects attributed to inhibition of the absorption of cholesterol from small intestines or the reabsorption of the bile acids, reduce cancer risk, immunity booster, reduced bone loss and antioxidant.

Triterpenoid acids serve significant pharmacologic activities such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer.

The insulin-like action of these bioactive compounds present in *M. oppositifolia* may be responsible for its hypoglycemic effects. Moisture content, ash content, and microbial determination test results of both tea forms are within acceptable levels (Table 1).

Table 3. Analysis of Variance on the anti-hyperglycemic property of the tea formulations grouped by type of treatment.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	193097.750	3	64365.917	8.473	.001
Within Groups	121544.800	16	7596.550		
Total	314642.550	19			

The tea products are within the range of 2.5% to 6.5% moisture content (Siddiqui *et al.*, 2013). Meaning that our products are less likely to deteriorate over time, thus our products have an increased shelf life. In terms of ash percentage, the products were well within the range of <5.54% (Siddiqui *et al.*, 2013).

Table 4. Post hoc Analysis of Variance on the blood glucose level of the diabetic test rats treated with *M. oppositifolia* tea treatments, treated with the commercial drug, and not treated.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Tea bag	Spray dried	-37.600	55.124	.925	-209.43	134.23
	Positive Control	-63.400	55.124	.727	-235.23	108.43
	Negative Control	187.200*	55.124	.030	15.37	359.03
Spray dried	Tea bag	37.600	55.124	.925	-134.23	209.43
	Positive Control	-25.800	55.124	.974	-197.63	146.03
	Negative Control	224.800*	55.124	.008	52.97	396.63
Positive Control	Tea bag	63.400	55.124	.727	-108.43	235.23
	Spray dried	25.800	55.124	.974	-146.03	197.63
	Negative Control	250.600*	55.124	.003	78.77	422.43
Negative Control	Tea bag	-187.200*	55.124	.030	-359.03	-15.37
	Spray dried	-224.800*	55.124	.008	-396.63	-52.97
	Positive Control	-250.600*	55.124	.003	-422.43	-78.77

*. The mean difference is significant at the 0.05 level.

This would mean that both the tea-bag and spray-dried tea have low levels of minerals which is the goal of tea because some high mineral content can lead to toxicity. The microbiological determination also showed that the products were within the reference given which are ≤107/g for aerobic plate count and ≤10MPN/g for the coliform count (American Diabetes Association, 2019). Similar to previous studies on herbal tea formulation wherein test results are within the prescribed limit (Okafor and Ogbobe, 2015; Kabilan *et al.*, 2019), the test results mean that the *M. oppositifolia* tea products are deemed clean and safe for public consumption. Table 2 shows the mean concentration of glucose in Sprague Dawley rats before and 12-hours after the administration of the different treatments and controls.

Table 5. Results of the paired t-test on the difference between the mean blood glucose concentration of Sprague Dawley rats before and 12-hours after the administration of treatment and controls.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Diff Teabag - Diff Spray-dried	-37.600	92.781	41.493	-152.803	77.603	-.906	4	.416
Pair 2	Diff Teabag - Diff Positive Control	-63.400	58.722	26.261	-136.313	9.513	-2.414	4	.073
Pair 3	Diff Spray-dried - Diff Positive Control	-25.800	87.902	39.311	-134.944	83.344	-.656	4	.547

The 12th hour after administration of any substance will account for the stabilization of chemicals and substances in the body that will account for a more reliable result (Whiting *et al.*, 2011). The results show that the teas were able to lower the concentration of blood glucose in hyperglycemic test animals. Table 3 shows that the F-statistic has a value of 8.473 with a p-value of 0.001 which is less than 0.05.

It speaks of evidence that there is varying effect between the different treatments as compared to the negative control.

Table 6. Results of paired t-test on the difference of the hypoglycemic effects between the *M. oppositifolia* tea-bag and spray-dried tea in Sprague Dawley rats.

Pairs	Mean	Std. Deviation	t-value	Sig. (2-tailed)
T1-T2	-36.933	64.962	-2.202^{ns}	.045
T1-T3	-18.60000	77.26651	$-.932^{ns}$	.367
T2-T3	18.33333	74.55072	$.952^{ns}$	.357

T1- Tea-bag, T2- Spray-dried tea, T3-Positive control.
Ns= not significant at 0.05.

This mean that there is indeed a significant difference on varying effect between the tea treatments as compared to the negative control. This is in contrary to the findings of the study by Ryan *et al.* (2000) that a native herbal tea failed to show an antidiabetic effect. In the clinical basis, the varying effect of the tea treatments may be due to the different mechanisms of the various metabolites present in the plant extract and the positive control in lowering blood glucose and the other kind of tea used. To check for the level of significance based on individual comparisons using a post hoc analysis, Scheffe test was used. Table 4 showed that the positive control, Glibenclamide, is still the most effective treatment at a 95% confidence interval.

Although it is worth to note that both the tea bag and spray-dried tea of *M. oppositifolia* greatly reduced the blood glucose level of the test animals and have no significant difference as the positive control.

Table 7. Summary of the characteristics of both teas according to the majority of respondents.

Characteristic	Tea-bag	Spray-dried tea
Color	Green	Green
Aroma	Herbal aroma	Herbal aroma
Taste	Bitter aftertaste	Bitter aftertaste

The positive control is a sulfonylurea product which works by preventing the production of glucose in the liver, improving the body's sensitivity towards insulin and reducing the amount of sugar absorbed by the intestines; while the teas contain various phytochemicals such as tannins, saponins and flavonoids which enhances insulin secretion and regeneration of Pancreatic β-cells and insulin-mediated glucose uptake by target cells. The findings are consistent with previous studies of herbal teas for diabetes showing that the glucose level is significantly lower in the tea treatment than placebo treatment, and no significant difference with the positive control (Jayawardena *et al.*, 2005; Chang *et al.*, 2013).

Further, Table 5 shows the result of the paired t-test on the difference between the mean blood glucose concentration of the test animal before and 12-hours after the administration of the two teas and the controls. It shows that all the various treatments are able to significantly reduce the concentration of blood glucose levels of the rat. Both the teas prove to be comparable with the positive control. But the spray-dried tea is better than the tea-bag. This may be due to the fact that in making the tea, the spray-dried powder can circulate through the boiling water. Studies show that powder is preferable because more phytoconstituents are retained. In line with the previous study by Eroglu *et al.* (2018), the disadvantage of tea bag is the inadequate brewing conditions which can be solved by using a powder tea. However, Table 6 shows that the difference between tea-bag and spray-dried tea is statistically insignificant which means that the effect of both teas is the same. Likewise, the results obtained when the tea-bag and spray-dried are compared with the positive control which is Glibenclamide, the data still are insignificant.

Table 7 shows a summary of the characteristics of both teas according to the majority. Both the tea-bag and spray-dried tea exhibit the same physical description that being that both of them are green in color, have herbal aroma with a bitter aftertaste. All in all, both teas exhibit the same sensory characteristics, as well as showing the characteristics expected of a tea. Comparing our results to those of older studies, it must be pointed out that the bitter aftertaste was least liked by the evaluators.

Conclusion

Formulations of *M. oppositifolia* in tea–bag and spray–dried powder teas have no significant difference as the commercial drug (Glibenclamide) in lowering the blood levels of hyperglycemic rats. Evaluation of moisture, ash, and microbial determination are all within the acceptable range.

It is also worthy to note than on a sensory evaluation of both tea forms have green color, herbal aroma, and bitter aftertaste.

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