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BLOOD GLUCOSE AND CHOLESTEROL LEVELS IN ALLOXAN-INDUCED DIABETIC MICE AFTER ORAL ADMINISTRATION OF SERPENTINA (*Andrographis paniculata*) AND PAPAIT (*Mollugo oppositifolia* L.) AQUEOUS EXTRACTS

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ABSTRACT

The effects of Serpentina (*Andrographis paniculata*) and Papait (*Mollugo oppositifolia* L.) aqueous extracts on blood glucose and cholesterol levels of 24 alloxan-induced diabetic 6-8 weeks old ICR male mice were investigated. The animals were distributed equally in a completely randomized design into three treatment groups with T1- Metformin at 500mg/kg, T2- Serpentina and T3-Papait at 400mg/kg BW PO. The substances were administered orally bid for 14 days. Blood glucose and cholesterol were determined using wet reagent chemistry. Blood glucose levels of all the animals in the treatment groups were greater than 200mg/dl, however T3 showed a consistent blood glucose lowering effect from day 0 to 14 causing 36.07% reduction compared to T2 which caused a fluctuating effect on blood glucose levels and a reduction of 22.53% while the animals in T1 showed the highest reduction at 45.29%. Also, animals in T3 at day 14 post administration (322.93 mg/dl) had comparable blood glucose levels (p>0.05) with T1 (284.50 mg/dl). No significant differences were observed in the cholesterol levels. Results showed that Papait has comparable blood glucose lowering activity with Metformin as compared to Serpentina.

Keywords: alloxan, blood glucose, diabetes, cholesterol, Serpentina

INTRODUCTION

Diabetes mellitus is one of the most common endocrine diseases of dogs and cats that may occur at any age. Despite the considerable progress in the management of diabetes mellitus by diet modification, traditional insulin treatment and use of synthetic drugs, the search for alternative products for the control of diabetes is going on (Ahmad and Qureshi, 2009) to look for natural, plant-based products that are cheap and readily available in the community.

Papait *(Mollugo oppositifolia linn)* and Serpentina *(Andrographis paniculata)* plants which are abundantly seen growing in plains, hill sides, farms, roadsides, and waste lands in the province of Isabela (Philippines) are used by the local folks for the management of diabetes mellitus. Papait is a diffuse prostrate, annual herb with slender and numerous dichotomously branced stems. The leaves appear in whorls of four to five and 13-22 mm long. Its flowers are small, white, in axial fascicles of two or more, with long filiform pedicels. Phytochemical constituents of Papait include alkaloids, flavonoids, glycosides, and phenolic compounds, phytosterols, saponins, tannins, triterpenoids, carbohydrates and proteins. The phenolic compounds and flavonoids in Papait were postulated to contribute to its antidiabetic activity by means of scavenging oxygen free radicals (Gopinathan and Subha, 2014).

Serpentina is a small annual shrub that grows erect to a height of 30–110 cm in moist and shady places with dark green slender stem, lance-shaped leaves with hairless

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blades measuring up to two cm long by 2.5 cm wide. Its leaves has been found to contain diterpenes, lactones and flavonoids (Kumar *et al.,* 2012). The plant is hypothesized to demonstrate an insulin stimulatory effect (Wibidu *et al.,* 2008) that is associated with its anti-diabetic activity.

Despite the different studies accounting for the antidiabetic potentials of these plants, no study has been conducted to compare the blood glucose and cholesterol lowering activities of these plants with each other and with Metformin. If proven successful, the results of the study can be considered as an alternative for Metformin in the management of diabetes mellitus.

MATERIALS AND METHODS

Twenty-four, six- to eight- week old Imprinting Control Region (ICR) male mice, weighing 20-25g from the Food and Drug Administration, Alabang, Muntinlupa City were used. The animals were acclimatized for one week and were subjected to conventional laboratory environment without control of humidity and temperature. All mice were fed pelleted diet (BMEG Pigeon pellet®) and given distilled water *ad libitum* daily. After the acclimatization period, diabetes was induced via intraperitoneal injection of Alloxan monohydrate (Sigma St. Louis, U.S.A.) as adapted from the study of Anunciado and Masangkay (2002).

Mature Serpentina and Papait plant leaves and stems were obtained from the local public market or farm land of Cabatuan, Isabela. The plants were identified and authenticated at the Botany Division Office, National Museum of the Philippines in Padre Burgos Drive, 1000 Metro Manila. The aqueous crude extracts of both plants were based on the methods used by Adedapo *et al.*, 2005.

All the animals were fasted for six to eight hours before blood sample collection through the ventral or lateral tail vein of mice. Blood collection and testing were done three days post alloxan induction which was monitored for 12 hrs and then at day 4, 7 and 14 thereafter. A drop of blood was placed on the blood glucose and cholesterol test strips (Easytouch® III Glucose and Cholesterol test strips, MHC Medical Products 8695 Seward Rd, Fairfield, OH, USA) and the strip was inserted on a glucose and cholesterol meter (EasyTouch® BPGC Meter, MHC Medical Products 8695 Seward Rd, Fairfield, OH, USA). The reading displayed on the screen was recorded.

The animals were distributed equally in a completely randomized design into three treatment groups. Each treatment was replicated twice with four animals per replicate. The treatment groups were as follows: T1- Metformin at 500mg/ dl, T2- Serpentina and T3-Papait at 400mg/ kg BW PO. The substances were administered orally using a gavage tube every 12 hours for 14 days. Reference for fixed 400 mg dose of Serpentina and Papait was adapted from the study of Hoque *et al.* (2011) and Zhang and Tan (2000).

All data were tabulated and expressed as mean ±SD, and analyzed using Analysis of Variance (ANOVA). Least Significant Difference (LSD) was used to determine the significant difference among group means at five (5) percent level of significance.

RESULTS AND DISCUSSION

Three days post-alloxan induction, the fasting blood glucose and cholesterol levels were measured in all the treatment groups. All animals from the three treatments were hyperglycemic with values almost two-times higher than 200 mg/ dl, the value to be exceeded in order to consider an animal as diabetic (Anunciado and Masangkay, 2002). Animals in T2 had the highest fasting blood glucose values (545 mg/ dl) followed

by T1 and T3 with mean values of 520 mg/ dl and 505.13 mg/ dl, respectively. These values were found to be comparable with each other. According to Rohilla and Ali (2012), alloxan selectively destroys the insulin-producing beta-cells found in the pancreas causing diabetes. Also, mean fasting blood cholesterol levels after alloxan induction showed that the animals in T2 showed the highest blood cholesterol values (144.63 mg/ dl), which was numerically higher but not significantly different with the values in T1 and T3 with 120.38 and 112.63 mg/ dl respectively. All animals in the three treatment groups showed hypercholesterolemia since the normal range of cholesterol level in mice is 26-82 mg/ dl as stated by Tully (2009). The mean fasting blood glucose and cholesterol values of all the treatment groups showed direct relationship of blood glucose and cholesterol values wherein increased blood glucose levels are accompanied by increased cholesterol levels. This may be due to the destruction of the insulin-producing beta cells in the pancreas by alloxan leading to permanent diabetes. In this case, the lack of insulin caused the peripheral cells not to take up additional glucose and therefore the animal's body utilizes alternative source of energy like fatty acids, ketones and amino acids. In this way, lipid cholesterol profile was increased (Reece, 2004).

Fasting blood glucose values of all the treatment groups three days after alloxan induction were monitored for 12 hours and plotted on a line graph to illustrate the blood glucose curve (Figure 1). Serial monitoring of blood glucose levels was performed to determine the dose and frequency of administration of a test substance or insulin necessary to keep the blood glucose levels on the normal or acceptable range.

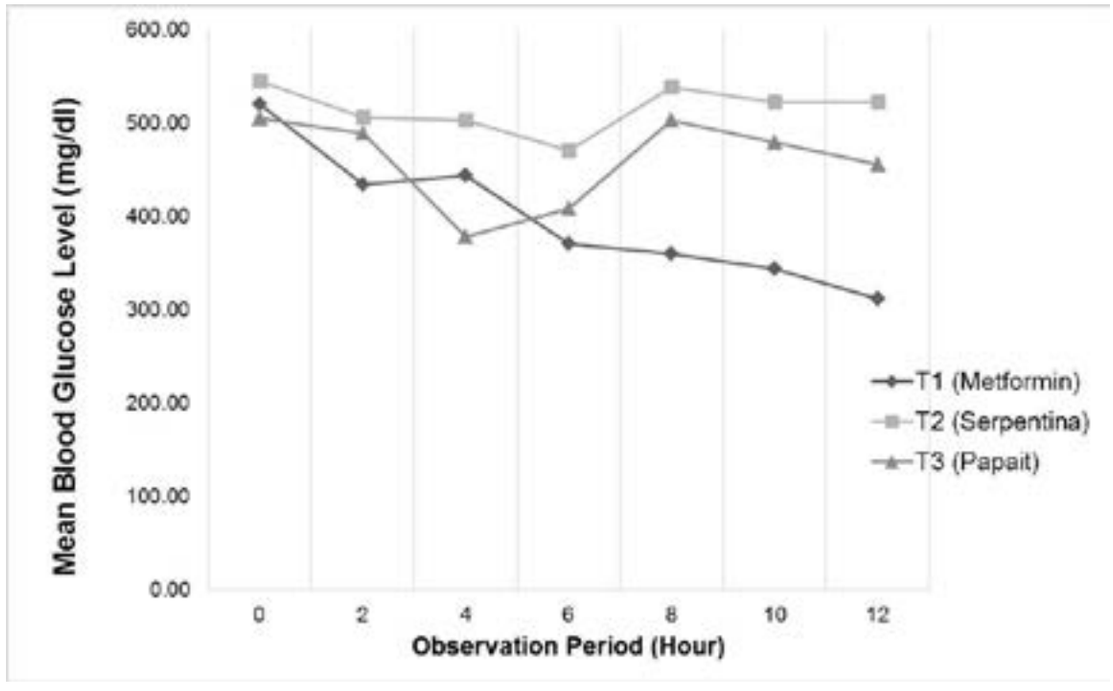

Figure 1. Blood glucose levels in alloxan-induced diabetic mice after oral administration of Metformin (T1), Serpentina (*Andrographis paniculata*) (T2) and Papait (*Mollugo oppositifolia linn*) (T3) aqueous extracts within 12 hours observation period.

It can be inferred from the graph that T3 had faster blood glucose lowering effect at the 4th hour post treatment with blood glucose values of 377.88 mg/ dl and after that, blood glucose levels again slowly increased peaking at the 8th hour post-treatment and slowly declined again until the 12th hour post-treatment. The 400 mg/ kg Papait (T3) of this study took its blood glucose lowering effect at the 4th hour post treatment which was slower compared to the crude methanolic extract results of Hoque *et al*. (2011), which recorded its lowest fasting blood glucose level at 90 min post-treatment. The blood glucose lowering effect of Papait may be explained by its role as an antioxidant enzyme which protects against free radicals that contribute in the oxidative stress by scavenging oxygen free radicals, caused by the pancreatic beta cell necrosis which released abundant free radicals. Antioxidants may also help strengthen the immune system of the animals which also contributes to its antidiabetic effect (Hoque *et al*., 2011).

Animals in Treatment 2 (Serpentina treated) had its lowest fasting blood glucose levels of 470 mg/ dl at the 6th hour post treatment and slightly increased with an average value of 538 mg/ dl at the 8th hour and a fluctuating slight decreased and increased for the succeeding hours of observation. The effect of T2 (Serpentina) in this study was slower compared to the ethanolic extracts of Serpentina result of Zhang and Tan. (2000) who reported suppressed elevated serum glucose level at 60 and 120 mins post treatment using 200 mg/ kg and 400 mg/ kg extract. Subramanian *et al*. (2008) also noted that Serpentina's antidiabetic activity acts by causing an increase in glucose utilization by delaying or preventing glucose absorption that are caused by the potential inhibitors of alpha glucosidase and alpha amylase that has been yielded in Serpentina plant. Also, the glucose lowering effect may be due to its insulin stimulating effect via ATP-sensitive potassium channels.

Metformin treated animals had a consistent blood glucose lowering activity for the 12-hours observation period with the lowest mean blood glucose values of 311.13 mg/ dl at 12 hours post treatment. In the study of Boyle *et al*. (2010), Metformin contributes an antihyperglycemic activity by activation of AMPK in skeletal muscle, liver and adipose tissue resulting in decrease circulating glucose, lipids, ectopic fat accumulation, as well as enhance insulin sensitivity, thereby regulating energy balance.

Table 1. Effect of the twice daily oral administration of Metformin (T1), Serpentina (*Andrographis paniculata*) (T2) and Papait (*Mollugo oppositifolia linn*) (T3) aqueous extracts on fasting blood glucose levels (mg/dl) ±SD in alloxan-induced diabetic mice within a 14-day observation period.

Treatment Groups	Treatment period (days)			
	0	4	7	14
T1 (Metformin)	520.00±77.11	427.94±185.93	452.86±111.07	284.50[a]±122.02
T2 (Serpentina)	545.00±60.73	471.50±84.47	522.14±59.88	422.21[b]±150.69
T3 (Papait)	505.13±135.23	461.75±159.83	373.43±241.28	322.93[ab]± 150.02

Note: Mean results with a different superscript are significantly different at P<0.05 (ANOVA, LSD).

Analysis of the mean fasting blood glucose levels as affected by the three treatment groups as shown in Table 1 revealed that the 14-day twice a day treatment with Metformin, Serpentina and Papait caused a decreasing trend of the fasting blood glucose levels, although all the animals in all treatment groups are still hyperglycemic.

Among the treatment groups, Metformin showed the highest percent reduction of (45.29%) of the mean fasting blood glucose level followed by Papait (36.07%) and Serpentina at 22.53% reduction.

The antidiabetic activity of Papait has been associated by Hoque *et al*. (2011) to its oxygen free radical scavenging activity. Serpentina aqueous extracts has also been reported by Akbar (2011) to possess increase activity of superoxide dismutase, catalase enzymes and hepatic gluthathione, substances that are also associated with antioxidant activity. Based on the results of this study, it can be inferred that the antioxidant activities of Papait might have affected better compared to that of Serpentina.

The lower blood glucose level percentage reduction of Serpentina might also be explained by one of its supposed mode of action to stimulate the release of insulin from pancreatic beta-cells through ATP-sensitive potassium channels (Akbar, 2011). In this case, alloxan induction caused permanent diabetes due to destruction of the beta-cells and therefore, it can cause available pancreatic beta cells to be stimulated are not present or if present in small number.

Table 2. Effect of the twice daily oral administration of Metformin (T1), Serpentina (*Andrographis paniculata*) (T2) and Papait (*Mollugo oppositifolia linn*) (T3) aqueous extracts on fasting blood cholesterol levels (mg/ dl) ±SD in alloxan-induced diabetic mice within a 14-day observation period.

Treatment Groups	Treatment period (days)			
	0	4	7	14
T1 (Metformin)	120.38 ± 15.31	115.25±12.91	129.36± 14.27	126.00± 12.31
T2 (Serpentina)	144.63±21.71	119.25±9.02	134.29±22.48	133.71±19.85
T3 (Papait)	112.63±16.95	115.75±9.69	129.86±13.01	129.50±9.29

No significant difference among the treatments on the fasting blood cholesterol levels was observed (Table 2) and it can be noted that the animals in all the treatment groups were hypercholesterolemic as well as hyperglycemic (Table 1).

Mean fasting blood cholesterol level among the treatment groups was observed to decrease on the 4th day of administration except for T3 which slightly caused an increase in cholesterol values. Mean cholesterol values at day 14 of the treatment regimen markedly increased in T1 and T3 at 126.00 mg/ dl and 129.50 mg/ dl, respectively, as compared to T2 which caused a decrease.

Only T2 (Serpentina) caused a reduction in fasting blood cholesterol levels at day 14 (7.55%), while T1 and T3 did not cause any decrease in the values, although animals in all the treatment groups are still hypercholesterolemic. According to Zhang and Tan (2000), Serpentina extracts can contribute a potential dyslipoproteinemia of the animals in STZ-diabetic rats. It showed that apart from carbohydrate metabolism, Serpentina extract can also play important role in lipid metabolism. The possible mechanism for decreased lipid levels could be either insulin releasing or insulin proved to inhibit the activity of hormone sensitive lipases in adipose tissue and suppress the release of lipids.

T3 showed a slight increase in cholesterol levels at day 14. This result coincides with the study of Gopinathan and Subha (2014) who reported reduced cholesterol levels only at day 35 post treatment with Papait. The high blood cholesterol levels in all the treatment groups can be associated to the high blood glucose levels in the animals. The serum cholesterol is elevated significantly in diabetes mellitus and other diseases. The decreased rate of lipid metabolism with diminished intestinal excretion of cholesterol and conversion of lipids into bile acids and other compounds frequently results in hypercholesterolemia (McDonald and Pineda, 1989).

Mean body weight values of all treatment groups declined at Day 14 with the lowest recorded body weight of 18.95 g in T3 causing 22.27% reduction, followed by T1

(21.07 g) with 11.28% reduction and T2 (22.14 g) with 6.78% reduction as compared to their initial mean body weight values of 24.38g in T3, followed by T2 and T1 with the same body weight of 23.75g (Fig 2).

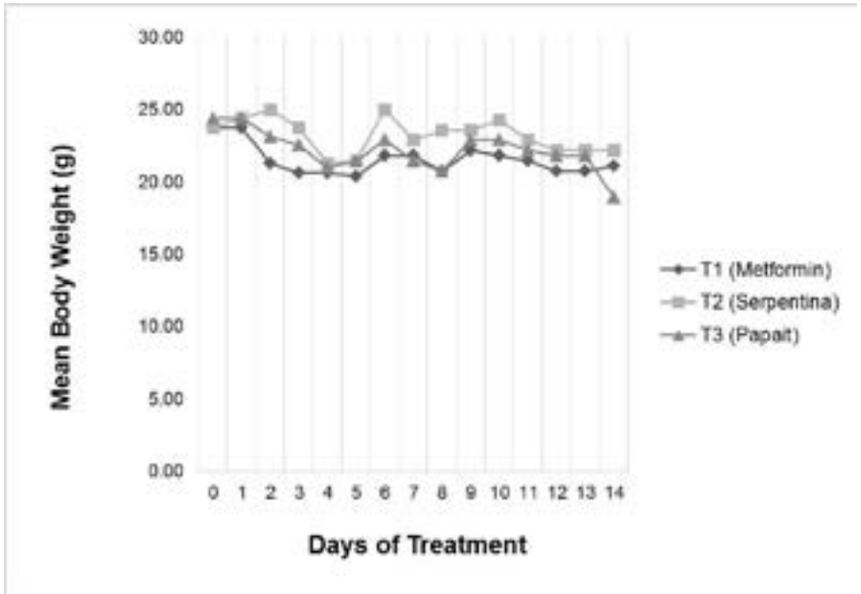

Figure 2. Mean body weight (g) of alloxan-induced diabetic mice after oral administration of Metformin (T1), Serpentina (Andrographis paniculata) (T2) and Papait (Mollugo oppositifolia linn) (T3) aqueous extracts within 14 days observation period.

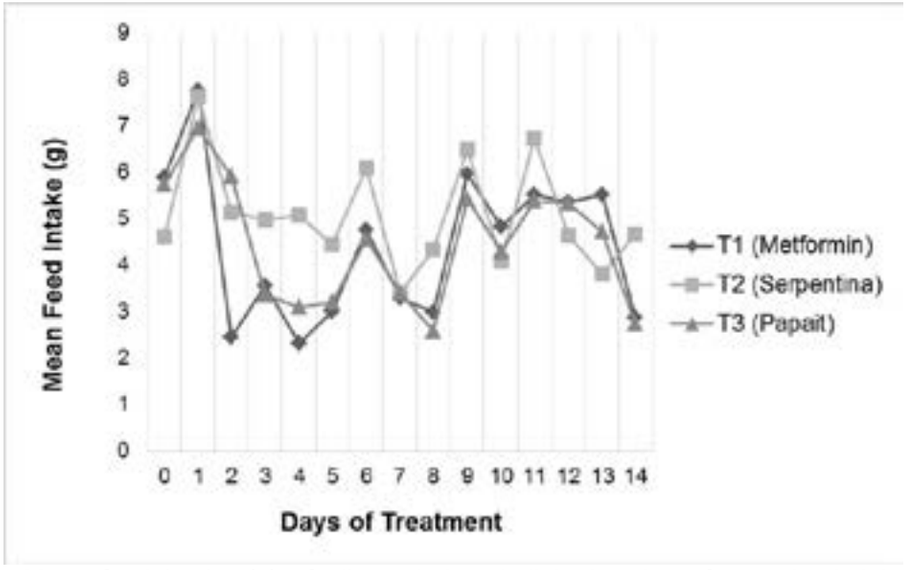

Figure 3. Mean feed intake (g) of alloxan-induced diabetic mice after oral administration of Metformin (T1), Serpentina (Andrographis paniculata) (T2) and Papait (Mollugo oppositifolia linn) (T3) aqueous extracts within 14 days observation period.

The weight loss observed in all the treatment groups is a common sequelae of overt cases of hyperglycemia due to the continuous loss of glucose in urine, wherein for every gram of glucose excreted, 4.1 kcal is lost (Anunciado *et al.,* 2004).

All treatment groups also showed a decreased in feed intake (Fig 3) with T3 causing the highest reduction in feed intake (52.36%) followed by T1, while T2 showed no reduction in feed intake. All the animals in this study did not exhibit a hyperphagic state which is normally seen in diabetic patients to cover up the caloric loss.

The prevention of the hyperphagic state in T3 may be attributed to the restoration of the normal circulating leptin levels of the animals. A slight increase in the feed intake of the animals in Treatment 2 does not coincide with the results of Zhang and Tan (2000), who recorded a decreased food and water intake of Serpentina treated animals.

CONCLUSION

Based on the results of the study, it can be concluded that Papait caused a more consistent decreasing pattern or trend of the fasting blood glucose levels of the animals, as compared with Serpentina, but showed comparable effects with Metformin although it was not able to normalize or cause fasting blood glucose levels below 200 mg/ dl if given twice daily for 14 days. Also, no significant effects on blood cholesterol levels, body weight and feed intake were observed. Further studies with longer duration should be conducted to find out the specific phytochemical compounds with antidiabetic activity present in these plants together with their mode of actions.

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