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

Karina Marie G. Nicolas¹, Kimberly M. Visaya², and Emmanuel R. Caunidad²

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 glucagon-like polypeptide-1, several plants have been used to alleviate the symptoms of diabetes mellitus. Although these plants have been documented to possess antidiabetic activities, there are very few studies that used the aqueous extracts of the leaves of these plants combined with conventional anti-diabetic medications. In this study, the anti-diabetic activities of the aqueous extracts of Mollugo oppositifolia L. (Papait) leaves and Andrographis paniculata (Serpentina) were compared with that of Metformin, an antidiabetic drug used to regulate blood sugar levels in diabetic patients. Papait was adapted from the study of Hoque et al., (2011) and Zhang and Tan (2000). Serpentina is a small annual shrub that grows erect to a height of 30–110 cm in dry, sandy soils or grassy areas. Its leaves have been found to contain diterpenes, lactones and flavonoids (Kumar et al., 2008) that is associated with its anti-diabetic activity.

MATERIALS AND METHODS

Twenty-four, six- to eight-week old 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. 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.

RESULTS AND DISCUSSION

Blood glucose and cholesterol were determined using wet reagent chemistry. 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/kg BW, 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.

Three days post-alloxan induction, the fasting blood glucose and cholesterol levels of all the animals in the treatment groups were greater than 200mg/dl, however T3 showed a consistent blood glucose lowering activity with Metformin as compared to Serpentina. 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. 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.

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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 blood glucose lowering activity with Metformin as compared to Serpentina.

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

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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 a 12-hours observation period.](Image)

There was a consistent decrease in blood glucose values in the Metformin treated animals. 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).

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 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 decreased circulating glucose, lipids, ectopic fat accumulation, as well as enhance insulin sensitivity, thereby regulating energy balance.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Treatment period (days)</th>
<th>0</th>
<th>4</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Metformin)</td>
<td>520.00±77.11</td>
<td>427.94±185.93</td>
<td>452.86±111.07</td>
<td>284.50±122.02</td>
<td></td>
</tr>
<tr>
<td>T2 (Serpentina)</td>
<td>545.00±60.73</td>
<td>471.50±84.47</td>
<td>522.14±59.88</td>
<td>422.21±150.69</td>
<td></td>
</tr>
<tr>
<td>T3 (Papait)</td>
<td>505.13±135.23</td>
<td>461.75±159.83</td>
<td>373.43±241.28</td>
<td>322.93±150.02</td>
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</tr>
</tbody>
</table>

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 (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 glutathione, 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.

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

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).
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.

ACKNOWLEDGMENT

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REFERENCES


