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Antidiabetic Evaluation of Pig (*Sus scrofa*) Bile on Alloxan-Induced BALB/C MICE

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Abstract

This study discerns to evaluate the antidiabetic efficacy of Pig Bile on Alloxan-induced BALB/c Mice. The experimental animals were divided and selected using RCBD into 5 groups (n= 4): T1 (negative control), T2 (1ml/kg), T3 (2ml/kg), T4 (3ml/kg) and T5 (Glibenclamide). Hyperglycemia was induced by injecting 1% alloxan monohydrate intraperitoneally. Glucose tolerance test was performed using 2g/kg glucose solution and blood glucose levels were measured at different time intervals. 14 days of monitoring was also done to ensure effectivity and efficacy of the different treatments. Body weight was also determined. Results shows that administration of treatments on test animals significantly reverted the blood glucose levels of mice in 60 minutes and 120 minutes using oral glucose tolerance test. After 14 days of monitoring, normal blood glucose levels were seen significantly on T2 (1ml/kg), T3 (2ml/kg), T4 (3ml/kg) and T5 (Glibenclamide) which only suggests the efficacy of pig bile on lowering glucose levels on alloxan-induced diabetic mice. Body weight analysis shows no significant difference. DMRT (Duncan's Multiple Range Test) shows comparable efficacy and effectivity between T4 (3ml/kg) and T5 (Glibenclamide) on lowering BGL at different day and time intervals.

Keywords: Pig bile, BALB/c Mice, Blood glucose

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Introduction

Through years of scientific advances in the field of medicine, newly-acquired knowledge paved way for the discovery of various treatments to medicate ailments and diseases. As a veterinarian, one is not concerned solely on the welfare of animals but also humans as well. Finding cure or prevention for diabetes in animals will not just primarily be confirmed on the concept of animal benefit but it may also serve as basis in formulating other drugs that could be used for humans. Canine diabetes is a common endocrine disorder with an estimated breed-related prevalence ranging from 0.005% to 1.5% in pet dogs. Increased prevalence in some breed suggests that diabetes in dogs is influenced by genetic factors and similarities between canine and human diabetes phenotypes suggest that the same genes might be associated with disease susceptibility in both species. Between 1-5% of human diabetes cases result from mutations in a single gene, including maturity onset diabetes of the adult (MODY) and neonatal diabetes mellitus (NDM) [1]. Although several synthetic drugs with antidiabetic effects including oral synthetic hyperglycemic agents like sulfonylureas group, insulin treatment given parenterally and specific enzymes inhibitors such as acarbose, miglitol are used. However, these drugs are expensive and commonly associated with side effects and drawbacks like insulin resistance, anorexia nervosa, brain atrophy, hepatotoxicity, abdominal pain, and flatulence which limits their applications [2]. Many of these oral antidiabetic agents have a number of serious adverse effects, thus, the management of diabetes without any side effects is still a challenge [3].

Traditional Chinese Medicine reported that, pig bile was shown to have anti inflammatory, anti-convulsion and analgesic effects. This could also prolong the survival time of mice under hypoxic conditions [4]. Keeping this in view, the work was undertaken to investigate the antidiabetic activity of bile content of Pig (Sus scrofa) bile that is readily available and found in the market of Tuguegarao City, Cagayan.

Materials and Methods

Collection of samples

The bile was obtained at the City Slaughter House, (Tuguegarao City, Cagayan) from an intact gallbladder of pig. After which it

was subjected to an autoclave to sterilize and ensure the safety of the experimental subjects. The bile was preserved to avoid contamination of foreign substances.

Experimental animals

Male BALB/c Mice were housed in Metabolic Cages and was fed with pellets and was given drinking water ad libitum. The animals were acclimatized for 2 weeks to their environment prior to experimentation proper. The animals were handled following the normal physiologic values of the mouse. The body temperature of 35.5-38ºC, at 6 months of age, weighing 20-40g which were obtained from United Doctors Animal Clinic and was housed at Cagayan Valley Herbal Processing Plant (CVHPP) Laboratory- Philippine Institute of Traditional and Alternative Health Care (PITAHC). All animals were supplied with standard pellet food and drinking water at 15 grams per day and 15 ml per day, respectively. Recommended environmental temperature of 65-85ºC and recommended environmental humidity of 30-70% and maintained under standard experimental conditions (temperature (28 ± 2) ºC; photoperiod: 12 hours light and 12 hours dark) throughout the experimental period.27 The normal blood glucose level of albino mice in male is 141 mg/dl or a range of 62-175 mg/dl.

Induction of Insulin-dependent diabetes mellitus

Alloxan solution was prepared by dissolving 1 g of alloxan powder in a normal saline which consist of 0.9 NaCl and 100ml of water. Diabetes was induced by intraperitoneal route of administration of 1 % alloxan solution to the BALB/c mice. The first phase that comes into view within the first minutes after alloxan injection is transcient hypoglycemic phase that lasts maximally for 30 minutes. The second phase appearing one hour after administration leads to rise in blood glucose concentration, this lasts for 2-4 hours. 3rd phase is again hypoglycemic phase that is noted 4-8 hours after injection. The last and the 4th phase of the blood glucose response is the final permanent diabetic hyperglycemic phase during which complete degranulation and loss of the integrity of the beta cells within 24-48 hours after administration of the alloxan takes place.

Experimental Design

The animals were selected using the Randomized Complete Block Design (RCBD) as showed on Figure 2, to eliminate the risk of bias. The animals were divided into five groups of three rats each, consisting of 2 control groups and three treatment groups. Treatment 1 as the negative control, diabetic mice administered with a distilled water as a placebo. Treatment 2 as a lowest dose, diabetic mice administered with daily oral of 1 ml/kg pig's bile. Treatment 3 as middle dose, diabetic mice administered with daily oral of 2 ml/kg pig's bile. Treatment 4 as highest dose, diabetic mice administered with 3 ml/kg pig's bile. Treatment 5 2 as the positive control, diabetic mice administered with standard drug (Alloxan Monohydrate, 10 mg/kg per day orally). Treatments of the Study Treatment 1 Negative Control (Placebo) Treatment 2 (1 ml/kg Pig's Bile PO) Treatment 3 (2 ml/kg Pig's Bile PO) Treatment 4 (3 ml/kg Pig's Bile PO) Treatment 5 Positive Control (Alloxan Monohydrate 10 mg/kg PO) Body weight of all animals was measured once before the treatment and twice after the treatment, on the 7th day and the 14th day end of studies. Fourteen days of administration of pig's bile will be given through the use of an oral gavage. Oral Glucose Tolerance Test On the 14th day of experimentation, the animals were made to fast for 12-14 hours but had free access of water and their fasting blood glucose level was measured four times. Glucose solution (2 g/kg of body weight) was administered orally in a volume of 1 ml/kg. Blood samples were collected 30 minutes after the administration of glucose at the29 time interval of 30 minutes, 60 minutes and 120 minutes after administration of treatments.

Statistical Analysis

Data's were expressed as a mean standard deviation. Differences among group treatment means were assessed by 2-factor analysis of variance (ANOVA) and group means were considered to be significantly different at $P < 0.05$ with Duncan's Multiple Range Test (DMRT) to analyze the level of significance between all treatment groups. Data will be statistically evaluated using Statistical Package for the Social Science (SPSS) version 20.0 software. Bar and line charts will be drawn using Excel 2016 software.

Data gathering

The data which will be gathered in the study are the following: 1. Mean and percentage of fasted blood glucose levels of each hyperglycemic alloxan-induced animals at different treatments post administration of pig bile and the control drug (Glibenclamide) at Day 0, Day 1, Day 7 and Day 14 of the study, also the statistical comparison of each treatment with the preceded day intervals. 2. Oral glucose tolerance of each hyperglycemic alloxan-induced animals measured at baseline, 30 minutes, 60 minutes and 120 minutes post administration of treatments, also the statistical comparison of each treatment with the preceded time intervals.30 3. Mean and percentage of body weight of each hyperglycemic alloxan-induced animals on Day 0, Day 7 and Day 14 of the study and statistical analysis.

Results and Discussions

Effects of different pig bile concentrations on fasted blood glucose

Alloxan-induced diabetic mice were treated with pig bile at different concentration, once a day, for 14 days. The effects of different doses of pig bile on fasting blood glucose level is presented in Figure 1. The present study was intended to examine the antidiabetic effects of pig bile at different doses. The dose of pig bile (1ml/kg, 2ml/kg and 3ml/kg) was selected based on the unpublished research . Alloxan monohydrate has been used to induce diabetes mellitus in experimental mice. A single intraperitoneal administration of 1 % alloxan monohydrate solution induced effectively diabetes mellitus in mice. This was confirmed by elevated level of fasting blood glucose that can be obtained from the tail of the mice after 48 hours of injection. Alloxan brings diabetes through selective destruction of insulin secreting pancreatic β-cells due to its accumulation through the glucose transporter 2 (GLUT2) and hence, minimize the glucose

uptake by peripheral tissues. It is known that alloxan induces free radical formation by redox reactions that cause tissue injury and make β cells to degranulate and consequently degenerate

Oral glucose tolerance test

The mean blood glucose levels of diabetic mice treated with placebo (negative control), diabetic mice treated with Glibenclamide (positive control) and diabetic mice treated with Pig bile at different concentrations were subjected to glucose tolerance test after two weeks is presented in Figures 1-3. The animals in each group (n = 4) fasted 12-14 hours and then fasting mean blood glucose level was evaluated after oral administration of glucose (2 ml/kg body weight) as a baseline.

This only says that the component of pig bile, specifically, cholic acid and chenodeoxycholic acid are natural ligands for the farsenoid X receptor (FXR) , and activation of FXR in liver may increase the production of small heterodimer partner (SHP) , a protein that plays a central role in lipid and glucose metabolism via regulation of various downstream molecules . The increase in SHP due to FXR activation increases glucose metabolism by inhibiting production of phosphoenol pyruvate carboxykinase (PEPCK), an enzyme associated with gluconeogenesis. Increase glucose metabolism can also be activated by G protein-coupled receptor 5 (TGR5)-cAMP-type 2 iodothyronine deiodinase pathway in brown adipose tissues. FXR activation also represses glucose levels in a diabetic rat model. Duncan's Multiple Range Test (DMRT) was used to further analyze the results between treatments w at different time intervals (Figure 4).

Body weight analysis on the effect on pig bile

The record of body weight at day 0, day 7 and day 14 is represented in Figure 5. The increase in body weight in the treatment group was shown by all the alloxan-induced mice although the increase was not significant when compared with the initial burden. The development of the body weight in all groups increased. Initial assessment of T2, T3 and T4 was significant with T5.

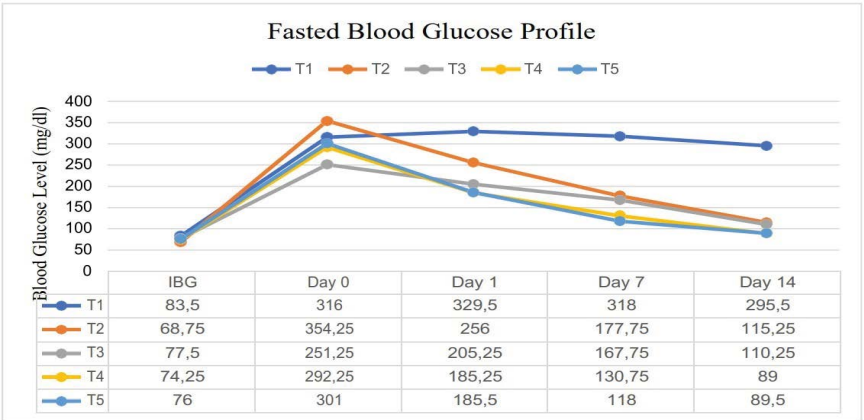
It can be inferred based on Figure 5, Treatment 2 (1 ml/kl), Treatment 3 (2 ml/kl) and Treatment 4 (3 ml/kl) shows a gradual increased in body weight as compared with Treatment 5 (Glibenclamide).

With this inference the present study agrees with Perino et al., 2014, that bile acids enter the bloodstream and behave like hormones, acting on receptors like TGR5, and affecting the behavior of different types of cells. TGR5 can block the chemical signals macrophages sends to attract more of their number into fat tissue. Also, liver X receptor (LXR), a glucose sensor, whose increased activity may increase insulin secretion from the pancreas and increase adipogenesis, improving adipose tissue functionality and decreasing its pathogenic tissue potential (adiposopathy) all leading to improved glucose sensitivity and improved glucose disposal. Overall, the antidiabetic activity of pig bile is due to the increased FXR activation and high production of SHP that inhibits gluconeogenesis which, in return activates the47 LXR in the liver and improved glucose sensitivity by preventing gluconeogenesis based on inhibition of the activity of PEPCK and G6Pase. LXR activation also improves glucose metabolism by promoting expression of glucokinase and glucose transporter 4 (GLUT4) in adipocytes and promotes insulin secretion in β cells in the pancreas and insulin sensitivity to tissues. Duncan's Multiple Range Test (DMRT) was used to further analyze the results between treatments with different treatments at different day intervals based on the observations on the change of body weight (Figures 6 and 7).

Summary

To compare the antidiabetic activity of different concentrations of pig bile in hyperglycemic alloxan-induced mice and to determine which concentrations (treatments) were more effective in lowering blood glucose level, three levels of pig bile concentration were made ranging from 1ml/kg-3ml/kg which were administered for 14 days to determine their fasted blood glucose [5].

Oral glucose tolerance test was also enforced to govern the amount of time for blood glucose to clear up from a hyperglycemic mice which were observed for 30 minutes, 60 minutes and 120 minutes post administration then were compared to the blood glucose level values to the test animals which received negative and positive control treatments. T1 (negative control) was treated with a placebo. T2 (1ml/kl) pig bile, T3 (2ml/kl), T4 (3ml/kl) and T5 (Glibenclamide) as the positive control drug [6].



	IBG	Day 0	Day 1	Day 7	Day 14
T1	83,5	316	329,5	318	295,5
T2	68,75	354,25	256	177,75	115,25
T3	77,5	251,25	205,25	167,75	110,25
T4	74,25	292,25	185,25	130,75	89
T5	76	301	185,5	118	89,5

Figure 1 Represents the mean blood glucose levels of hyperglycemic mice post administration of treatments at different day intervals.

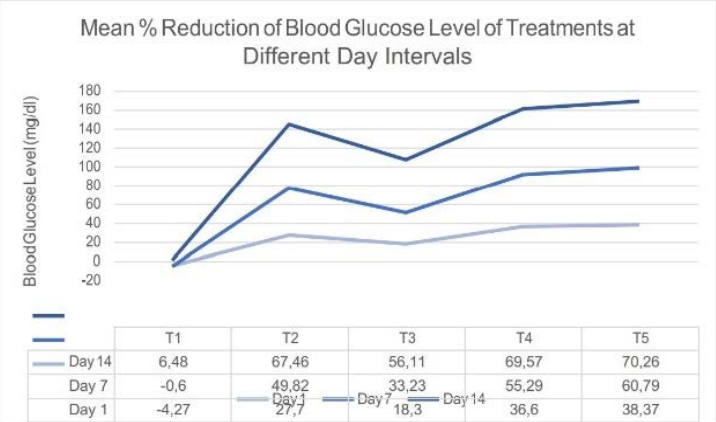


Figure 2 Represents the mean percent reduction of blood glucose levels of the different treatments at different time intervals (Day 1, 7 and 14). Same pattern of low to high percent reduction was gleaned at T1 (Negative Control), T2 (1ml/kg), T3 (2ml/kg), T4 (3ml/kg), T5 (Positive Control). It can be concluded that the treatments are working effectively on lowering the blood glucose level of.

Effect	SS	df	MS	F	p
Days	294828	3	98276	41.677	0.000000
Treatments	230756	4	57689	24.465	0.000000
Variation between Days and Treatments	77247	12	6437	2.730	0.005171
Error	141482	60	2358		

Duncan's Multiple Range Test (DMRT) was used to further analyze the results between treatments with at different day intervals. (Appendix Table 2-7).

Figure 3 Two factor Analysis of Variance showed that there is a significant difference on the days and treatments of fasted blood glucose at $p < 0.05$ and even at $p < 0.05$ and even at $p < 0.01$ level of significance.

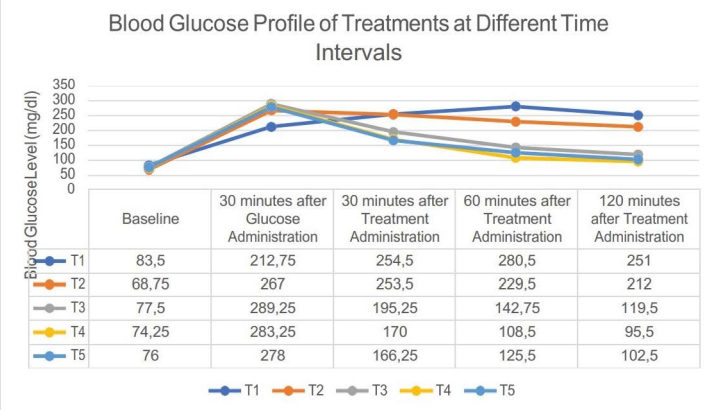


Figure 4 Oral glucose tolerance test.

Effect	SS	df	MS	F	p
Days	390443	4	97611	785.24	0.000000
Treatments	84873	4	21218	170.69	0.000000
Variation between Days and Treatments	129191	16	8074	64.96	0.000000
Error	9323	75	124		

Figure 5 Two factor Analysis of Variance showed that there is a significant difference on the time intervals and treatments of fasted blood glucose at $p < 0.05$ and even at $p < 0.05$ and even at $p < 0.01$ level of significance.

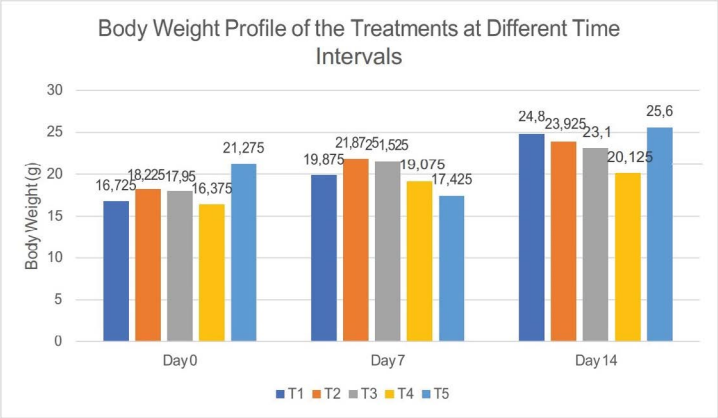


Figure 6 Body Weight Profile of the Treatments at Different Time Intervals.

Effect	SS	df	MS	F	p
Days	299	2	149.5	15.376	0.000000
Treatments	166.24	2	41.56	4.274	0.005130
Variation between Days and Treatments	27.77	8	3.47	0.352	0.937493
Error	437.53	45	9.72		

Figure 7 Two factor Analysis of Variance showed that there is a significant difference on the time intervals and treatments of fasted blood glucose at $p < 0.05$ and even at $p < 0.05$ and even at $p < 0.01$ level of significance.

The data gathered were mean and percentages of fasted blood glucose levels of each hyperglycemic alloxan-induced animals at different treatments post administration of pig bile and the control drug (Glibenclamide) at Day 0, Day 1, Day 7 and Day 14 of the study, also the statistical comparison of each treatment with the preceded day intervals. Oral glucose tolerance of each hyperglycemic alloxan-induced animals measured at baseline, 30 minutes, 60 minutes and 120 minutes post administration of treatments, also the statistical comparison of each treatment with the preceded time intervals and mean and percentage of body weight of each hyperglycemic alloxan induced animals on Day 0, Day 7 and Day 14 of the study, also percent reduction of fasted blood glucose and oral glucose tolerance was also seen together with the percent increase in body weights of the animals [7-10].

Based on the mean and percentage of fasted blood glucose of treatments at different day intervals, results showed that T2 (1mg/kg), T3 (2mg/kg) and T4 (3mg/kg) subsequently lowers down blood glucose levels with mean percent reduction of 67.46 %, 56.11 % and 68.57 % at day 14 as compared with the positive control at 70.26 percent reduction, respectively. This clearly shows the effectiveness of different concentrations of pig bile on lowering the blood glucose level of the animal. The study showed that there is no mortality caused by the different concentrations of pig bile indicating their highest safety level. Statistical analysis showed no significant difference at $p < 0.05$ percent and even at $p < 0.01$ percent. DMRT reveals treatments with significant differences among treatments at different day intervals [11].

Analysis of blood glucose reduction levels in mg/dl were noted at 30 minutes, 60 minutes and 120 minutes post administration

of different treatment concentrations of pig bile subsequently there were eight trials in the study period. The data showed that all treatments has a reduction in blood glucose levels with T4 (3ml/kg) as the highest followed by T3 (2ml/kg) and T2 (1ml/kg) at 66.28 %, 58.68 % and 20.59 %, respectively at 120 minutes as compared with T5 (positive control) with 63.12 % reduction [12-15]. Statistical analysis showed no significant difference at p<0.05 percent and even at p <0.01 percent. DMRT reveals treatments with significant differences among treatments at different time intervals. Results of percent increase in body weight shows significant difference among all treatments [16-20].

Conclusions

The results of the study provide evidence that:

1. The pig bile has antidiabetic effect on hyperglycemic alloxan-induced BALB/c mice.
2. Concentrations of T2 (1ml/kg), T3 (2ml/kg) and T4 (3ml/kg) were effective in lowering blood glucose level with 67.46 %, 56.11 % and 68.57 % mean percent of reduction. Statistical analysis showed no significant difference at p <0.05 percent and even at p <0.01 percent.
3. Concentrations of T4 (3ml/kg) as the highest followed by T3 (2ml/kg) and T2 (1ml/kg) at 20.59 %, 58.68 % and 66.38 % respectively, shows efficacy and effectivity on lowering blood glucose clearance at 120 minutes as compared to T5 (positive control). Statistical analysis showed no significant difference at p <0.05 percent and even at p <0.01 percent.
4. Mean percentage increase of body weight shows significant findings among all treatment.

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