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Effect of insulin (SciLin M30) and metformin in the growth and development of a chick

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Abstract The effect of insulin (SciLin M30) and metformin in the growth and development of a chick was examined through experimentation in 16 days. The results showed that metformin decreased the rate of morphological growth of chick and affected the development of the kidney. Thus, the dosage intake of metformin should be appropriated with the age. Meanwhile, insulin (SciLin M30) decreased the gain of weight during the growth of a chick. The feather buds of body and wings, and color variation were fairly observed in all the treatments.

Keywords: Insulin, Metformin, Growth, Development, Chick

Introduction

Medicine is a form of healing that quest for health and wellness of the body system. Health is a condition or state of the body which requires observation and awareness to avoid sicknesses and to maintain good stamina. Different medicines appear in the market that comprises organic and inorganic drugs monitored and checked by the Department of Health. These are provided to give necessary way to protect and keep oneself from detrimental factors occur in the environment, food and water intake. Supplements and medicines provide nutrients and help maintain resistance that ultimately affects the condition of the body. The importance of medicines is reflected in the study of pharmacology, which encompasses not only treatment by drugs, diet, exercise and other surgical means but also to maintain our health to prevent disease, injury and other damage to a body or mind (Alinio, 2007).

Diabetes mellitus is a major growing public health problem (Skyler, 2003) which has reached epidemic proportion throughout the world (Datar and Bhonde, 2011). It is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (Diabetes Care, 2009). Different media were formulated to regulate the hormonal secretion of insulin to absorb glucose from the blood that the body cells need for metabolism.

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Insulin-lowering agents reduce too much insulin secretion in the body. The lifestyle changes and treatment with metformin reduced the incidence of diabetes in persons at high risk (Knowler *et al.*, 2002). In relation, various preparations of insulin (SciLin) are formulated to provide short, intermediate and biphasic therapeutic action in which the blood glucose is maintained with a reasonable range. The agents are necessary to maintain the amount of insulin in human body. However, side effects may occur if not properly identified and observed.

Human needs supplements to maintain a normal body condition and to avoid failure of different body organs. The formulations of the two anti-diabetic agents are needed to normalize the insulin production. The contact of these agents may give negative and positive effects in the body. The study aimed to determine if the agents such as Insulin (SciLin M30) and Metformin have effects to the external (morphometrics and weight) and internal organs of the body of a chick during the development in an effort to relate the efficacy of these agents to human development. It is important to use experimental model in order to uncover the mechanisms which participate in the initiation and progression of body lesions (Ayala *et al.*, 2006) and to evaluate pharmaceutical agents and drugs. Thus, the purpose of this study was to evaluate the effect of insulin and metformin in the growth and development of a chick.

Materials and methods

Experimental animal

Various laboratory experiments used animals as medium of showing visible and providing scientific proof towards inorganic and organic factors. Due to the development of experimental strategies and methods, researchers established interests in the field of developmental biology using animals. The work with experimental animals has led to information on the structure of nutrient-containing compounds through metabolic and digestive processes (Baker, 2008). Hence, chicken is one of the popular model systems known for its experimental advantages (Mok *et al.*, 2015). It is usually served as the representative of avian group in experimental studies (Ainsworth *et al.*, 2010). Chicken egg is an attractive model which continuously gives major contributions in understanding molecular and cellular mechanisms that control developmental processes (Mok *et al.*, 2015). For more than two millennia, its rapid development and accessibility for visualization and experimental manipulation are some characteristics that made it a vertebrate model of choice (Vergara and Canto-Soler, 2012). It has been described as the premier non-mammalian vertebrate model organism which represents a significant component of the world's food supply (McDonnell and McDonnell, 2014).

In fact, the sequence of chicken genome was released to boost applications and research in medicine and agriculture (Burt, 2004) like human health and animal health, respectively. Many of the major concepts of developmental biology, such as induction, plasticity, competence and contact inhibition, are due to work done on the chick (Vergara and Canto-Soler, 2012). In addition, through transgenic chick model, it will benefit studies on embryonic development as well as providing economical bioreactor for pharmaceutical industry (Chapman *et al.*, 2005).

Experimental protocol

There were 34 chicks used in the experiment. The twenty-four chicks were randomly divided into four experimental treatment groups with six chicks each. The chicks were placed in a cage with controlled temperature to maintain the body condition. Group 1 was the control group; the group 2 samples were injected orally within 16 days with 10ml of dextrose, group 3 samples were treated daily within 16 days with 10ml of Insulin (SciLin M30) and the samples of group 4 were treated with 7mg of Metformin within 16 days. The remaining ten chicks were treated with 125 mg of Metformin for further observation. Each of the treatment was labeled for identification. Chicks were equally fed with 25 grams of chicken feeds everyday for sixteen days. The chicks were morphologically and anatomically examined to identify the effect of the agents in the entire growth and development.

Morphometrics and weight of the samples of each group were recorded to observe the changes during the growth of the chicks. Initial metric variables were gathered before the treatment.

Dissection of one chick from each group was performed to measure the length of the selected internal organs such as heart, kidney and liver. Protocol in laboratory dissection was followed during the collection of data. Lengths of heart, kidney and liver were gathered using vernier caliper (in cm) during day 0 (initial) and day 16 (final) only. The internal (anatomy) and external (morphology) characteristics were recorded in each treatment in terms of color for further comparison. The internal characteristics were evaluated during dissection.

Experimental analysis

The study used mean average for the ornithological measurement, body measurement, weight, and wingspan. The percentage length and weight gain were computed by using the formulas below:

% Length Gain= [(Final Length – Initial Length) / Initial Length] x 100

% Weight Gain= [(Final Weight – Initial Weight) / Initial Weight] x 100

Results

The results of the data analysis of the ornithological measurement growth and percentage length gain of chick groups subjected to various agents (dextrose, insulin and metformin) are presented in Table 1. After 4 days, the metformin treated chicks showed the lowest growth average than the rest of the treatments including the control group followed by insulin treated chicks. Similarly in day 8, metformin treated chicks showed the lowest growth average followed by the insulin treated chicks. From Day 12 to Day 16, the metformin treated samples were the lowest growth response followed by the insulin compared with the normal increase response in control group. In the percent length gain, dextrose and insulin treated chicks were higher than the normal percentage of growth of the control group. It means that the dextrose and insulin had a good effect in the growth of the chicks. However, the metformin treated group had a negative effect in the growth and development of the chicks, implying that the use of metformin decreases the rate of growth of the treated samples.

Table 1. Average ornithological length and percentage ornithological length gain of chick groups under different agents in 16 days

Average (in cm)	Control	Dextrose	Insulin (SciLin M30)	Metformin
Day 0 (initial)	11.95	11.40	11.65	11.43
Day 4	13.72	13.74	13.50	13.48
Day 8	14.55	14.70	14.35	13.90
Day 12	14.80	15.45	14.80	14.77
Day 16 (final)	16.20	16.15	16.05	14.93
% Length Gain	35.56	41.67	37.77	30.62

% Length Gain= [(Final Length – Initial Length) / Initial Length] x 100.

Data of the average body measure and percentage body measure gain of the chicks after 16 days of treatment under different agents are presented in Table 2. The group treated with metformin had the lowest average compared to the three groups. Hence, the growth in this group was below the normal condition of changes. The two treated groups together with the control had reached almost close average value. In day 12, the three groups namely control, dextrose, and insulin had the same average measurement and metformin group remains the lowest average. In addition, day 16 showed that metformin group had lowest average increase of body measurement compared to the control group. The

percentage body measure revealed that the insulin can cause body enlargement in two weeks. However, metformin group showed that the agent had negative effect in normal body growth and development.

Table 2. Average body measure and percentage body measure gain of chick groups under different agents in 16 days

Average (in cm)	Control	Dextrose	Insulin (SciLin M30)	Metformin
Day 0 (initial)	9.75	9.58	9.15	9.45
Day 4	10.40	10.16	10.18	9.82
Day 8	10.56	10.35	10.20	9.93
Day 12	11.30	11.30	11.30	10.70
Day 16 (final)	11.90	12.08	12.35	11.13
% Body Measure Gain	22.05	26.10	34.97	17.78

% Body Measure Gain= [(Final Body Measure– Initial Body Measure) / Initial Body Measure] x 100.

Group of chicks average weights are presented in Table 3. Gradual increased in weight had happened which is directly proportional to ornithological and body measurement growth. In day 4, dextrose treated group had the highest average, denoting that the dextrose increases the rate of development and body strength resulting to gain weight. The two remaining treatments were almost close to the average weight of the control. From day 8 to day 16, metformin treated groups remained the lowest average weight compared to the control group. Moreover, average weight of insulin treatment group remained lower than the control group. In metformin group, the average weight in the day 12 decreased in the day 16. Similarly, the insulin treated group decreased the rate of weight gain but not as the rate of the metformin group. The percent weight gain showed that metformin and insulin groups had lower percentage compared to the control group where metformin group had the lowest percent gain. In addition, dextrose group remained the highest in gain percentage.

Table 3. Average weight and percentage weight gain of chick groups under different agents in 16 days

Average (in cm)	Control	Dextrose	Insulin (SciLin M30)	Metformin
Day 0 (initial)	37.74	37.25	37.13	37.50
Day 4	38.80	41.00	38.40	38.00
Day 8	50.00	53.00	45.25	44.00
Day 12	65.50	76.25	67.00	64.33
Day 16 (final)	79.00	85.00	77.00	63.33
% Weight Gain	109.33	128.19	107.38	68.88

% Weight Gain= [(Final Weight – Initial Weight) / Initial Weight] x 100.

Average wingspan lengths are presented in Table 4. During the initial day, the wingspans of the samples were measured. In insulin group, the averages of the wingspans were lower than the control group from day 4 to day 12 but in day 16, there was a sudden increased in length. The wingspan averages in metformin treated group were lower than the normal averages of the control group. The dextrose treated group wingspan averages were consistently higher than the control group. However, the length gain of insulin treated group had the highest percentage attained in 16 days which had an abrupt increase in last day. However, metformin treated group had the lowest percentage length gain.

Table 4. Average wingspan and percentage wingspan gain of chick groups under different agents in 16 days

Average (in cm)	Control	Dextrose	Insulin (SciLin M30)	Metformin
Day 0 (initial)	12.78	12.98	12.48	12.38
Day 4	12.98	13.34	12.62	12.46
Day 8	13.70	13.75	13.30	13.47
Day 12	13.80	15.45	13.47	13.49
Day 16 (final)	16.10	16.55	16.85	14.50
% Length Gain	25.98	27.50	35.02	17.12

% Length Gain= [(Final Length – Initial Length) / Initial Length] x 100.

Chick heart metrics are presented in Table 5. During the initial day of exposure to agents, all chicks were recorded with the same heart length. The measurement of the heart length was from the aorta to the vertical end part. Data showed that the increase in lengths in all the groups was the same. Hence, there was no effect of the agents in the development of the heart.

Table 5. Heart length of chick groups under different agents in 16 days

Average (in cm)	Control	Dextrose	Insulin (SciLin M30)	Metformin
Day 0 (initial)	16	16	16	16
Day 16 (final)	21	21	21	21

Liver lengths of the chicks in each day (initial and final) are presented in Table 6. All chicks were recorded with the same liver length. In the final day, the same measurements were recorded in each group. Data showed that the agents had no effect in the enlargement of the liver.

Table 6. Liver length of chick groups under different agents in 16 days

Average (in cm)	Control	Dextrose	Insulin (SciLin M30)	Metformin
Day 0 (initial)	21	21	21	21
Day 16 (final)	27	27	27	27

Lengths of the kidney are presented in table 7. Initial lengths were recorded in all the groups having the same measurement. In day 16, dextrose and insulin treated chicks had the same kidney lengths which means that the agents had no effect in the growth and development of the kidney. However, the metformin treated group showed otherwise.

Table 7. Liver length of chick groups under different agents in 16 days

Average (in cm)	Control	Dextrose	Insulin (SciLin M30)	Metformin
Day 0 (initial)	6	6	6	6
Day 16 (final)	10	10	10	8

Qualitative data

In 16 days of treatment, agents such as dextrose, insulin (SciLin) and metformin showed no effect in the development in the three treated groups in terms of feather and beak. Permanent feather buds started to grow in all parts of the body including the wings feather. Colors of the wings of each treated chicks were visible and color variation in the feather was visible at the final day of the observation. Hence, all the changes happened in the morphological characteristics were fairly seen in all the samples in each group. In addition, the selected organs such as heart, liver and kidney showed the same complexion. No lesions were diagnosed in the selected parts of the internal organs. It means that the insulin and metformin had no effect in terms of quality and characteristics.

Dosage of metformin

In the development of a one-day old chick, proper dosage of agents should be administered orally. In the initial day, 125mg of metformin has been orally injected. After 3 hours, the injected chicks died. The internal body organs of the chick cannot contain large amount of agent with strong chemical content. In the same way, there is always a required dosage for the intake of such drugs.

Discussion

It has been known that chicken is a widely used model in embryonic development as a representative of vertebrates in all aspects. Hence, some researchers used it to counterpart human for laboratory and medical experiment. As evidence, chickens have similar concentrations of circulating insulin compared with mammals maintaining high plasma glucose levels (Dupont,

2009). According to Simon *et al*. (2012), chickens can mimic an insulin-resistance state by exhibiting peculiarities with regard to its insulin control and glucose level in plasma. In the year 2008, Dupont *et al.* evaluated the role of insulin in chicken using insulin immune-neutralization. It was observed that insulin signaling in muscle is peculiar in chicken and is strictly dependent on insulin in fed status. Moreover, typical insulin receptors are present in chicken kidneys (Bisbis *et al.*, 1994). Hence, insulin receptors have been characterized in a cell line isolated from a chicken hepatoma (Taouis *et al.*, 1993).

The administration of appropriate doses of insulin to patients with diabetes mellitus, along with controlled diet and exercise, temporarily restores their ability to metabolize carbohydrates, fats and proteins; to store glycogen in the liver; and to convert glucose to fat. When given to a diabetic patient at appropriate doses and dosage intervals, the blood glucose is maintained within a reasonable range, the urine remains relatively free from glucose and ketone bodies, and diabetic acidosis and coma are prevented.

Metformin, widely given to type 2 diabetic patients, induces muscles to take up glucose from the blood resulting to the reduction of risk of cancer (Evans *et al.*, 2005). Its early use in treatment algorithms is supported by lack of weight gain, low risk of hypoglycaemia and its mode of action to counter insulin resistance (Scarpello and Howlett, 2008). In body weight reduction, it indicates that the use of it decreases the intake of calorie in a dose-dependent manner leading to weight loss (Lee and Morley, 1998). Metformin decreases insulin resistance and gives beneficial effect because insulin promotes cancer cell growth (Sahra *et al.*, 2010). Metformin is a strong therapy for delaying the onset of the disease as part of a treatment programme to correct features of the metabolic syndrome (Bailey, 2007). It decreases the amount of blood sugar that the liver produces and that the intestines or stomach absorb (Nasri *et al.*, 2014) and has also been shown to have several beneficial effects on cardiovascular risk factors (Hundal and Inzucchi, 2003).

Pregnancy increases requirements for insulin secretion while increasing insulin resistance (Glueck *et al.*, 2003). The use of metformin therapy in hypertensive and gestational diabetes may have beneficial effects on pregnancy loss and development of pregnancy-related problems (Kumar and Khan, 2012). Furthermore, use of this has no demonstrable teratogenic effects, developmental delays or intra-uterine deaths (Lautatzis *et al.*, 2013). However, there is a genuine risk of the accumulation of it and associated lactic acidosis in chronic kidney disease (Herrington *et al.*, 2013). Therefore, it should be remembered that the benefits of metformin intake do not extend to the renal system (Bombardier *et al.*, 2008).

Based on the result of the study, with a chick as an experimental animal, the exposure of the developing organism to different drugs, with metformin and insulin as examples, can interfere the overall development. It was manifested in the mortality rate, morphological and anatomical measurements, and conditions of the selected organs (heart, kidney and liver) that metformin has severe effects, and less for the insulin. Thus, it is recommended that the intake of metformin during human pregnancy should be lessened or regulated. In order to further determine the effect, it is also recommended to examine the chick exposed to metformin and insulin at histological and cellular level.

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