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Anti-diabetic and hypolipidemic effects of raw quail (*Coturnix japonica*) eggs on alloxan induced diabetic rats

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Abstract

This study evaluates the anti-diabetic activity Quail egg in an alloxan induced hyperglycaemia in rats, using chlorpropamide as a standard oral anti-diabetic drug. The anti-diabetic effect was evaluated using 60 rats. splatted to 5 groups of 12 rats, consisting of normal control, diabetic rats treated with Quail egg, diabetic control rats, chlorpropamide treated rats (84mg/kg) and normal rats treated with Quail egg, once daily, orally. Diabetes was induced using 100mg/kg body weight of alloxan. The pancreas was examined histopathologically. The serum level of glucose of the rats was evaluated after 24 hours, to confirm diabetes induction, with subsequent treatment with quail egg and chlorpropamide, for 49 days. Serum glucose and lipid profile (cholesterol, triglycerides, LDL and HDL), were determined after the 49^{th} dose of treatment with quail egg and chlorpropamide. Following treatment with quail egg and chlorpropamide, once daily, for 49 days, a significant hypoglycemic and hypolipidemic effect was observed as evident from lower serum levels of the glucose and lipid profile, when compared to diabetic control group. Nonetheless, significant higher (P<0.05) mean serum level of glucose as well as lipid profile were observed in diabetic rats treated with Quail egg than in normal control. Histopathological examination of pancreas of diabetic rats treated with the quail egg shows low percentage regeneration of β -cells. It can be concluded that Quail egg has low antidiabetic and hypolipidemic effects and may therefore not confer benefit when used as a single antidiabetic agent.

Keywords: Diabetes, Quail egg, Blood sugar, Lipid profile

1. INTRODUCTION

Non-communicable diseases (NCDs) are greatly avoidable, but sadly account for 71% of death globally (Toniolo et al., 2019). Among the NCDs, diabetes is

one of the major, accounting for almost 1.6 million premature deaths (Toniolo et al., 2019). Diabetes mellitus (DM) according to International Diabetes Federation is a multifactorial metabolic disorder linked with chronic hyperglycemia, which arises due to relative or absolute insulin deficiency in the human system. Almost all cell types in the body, are damaged by chronic hyperglycemia. Diabetes is linked with acute and chronic complications, diabetic ketoacidosis and non-ketotic conditions. Uncontrolled diabetes impaired blood vessels and nerves leading to various conditions; blindeness (retinopathy), stroke, kidney and heart failure (Harding, Pavkov, Magliano, Shaw, & Gregg, 2019). Origin and etiology of diabetes occur either inform of insulin secretion defects and response or their mixture. High numbers of patient with DM are contacted with Type 1 (immune-mediated) or Type 2 (non-insulin dependent DM). In this regard, the most common is Type 2 DM with features of persistent hyperglycemia, insulin resistance as well as relative insulin deficiency. Diabetes also can be linked to certain drugs, gestational hormonal environment, genetic defects and infections (Wondifraw, 2015).

The growth of diabetes has been rising dramatically in recent years. It is documented that 537 million adults with average age from 20 to 79 years are contracted with type 2 DM accounting about 90% of the cases globally. Currently, it is estimated that over 24 million people are diabetic patients in Africa with almost 62% of non-diagnostic patients and the number is expected to rise up to 41.1 million by 2023. In Nigeria, it is documented that about 3.9 million people are diabetic patients with prevalence of 4.99 % (Dahiru, Aliyu, & Shehu, 2016). Moreover, all nation regions are affected with the higher prevalence rate of 9.8 % in South-south region and lower rate of 3% in northwest zone (Uloko et al., 2018). Diabetic patients need a lifelong management to successfully control diabetes. It negatively affects not only the patient health, but also the health of families, community, the economy and the targeted goals of international organization such as sustainable development goals (SDGs) (Harding et al., 2019; Oguejiofor, Onwukwe, & Odenigbo, 2012). Eggs can make remarkable contribution to a healthy meal. Studies reveal that consistent egg consumption yield better diet quality, as a survey data in American shows that egg consumers have all nutrients except dietary fiber and vitamin B6 compared to those not consuming eggs. Cholesterol and saturated fat content of egg, makes it a controversial food for health agencies and nutritionist (Li, Zhou, Zhou, & Li, 2013). This was based on the believe that high dietary cholesterol intake result in high blood cholesterol level and cardiovascular diseases (Eilat-Adar, Sinai, Yosefy, & Henkin, 2013), but was found to be lower among frequent consumers of egg (Song & Kerver, 2000).

Quail (Coturnix japonica) eggs with their small size have nutritional value, three to four times more than chicken eggs as it is rich in antioxidants, minerals and vitamin (Tunsaringkarn, Tungjaroenchai, & Siriwong, 2013). Age, diet, environmental as well as strain can affect the composition of quail egg (Fraeye et al., 2012). Quail eggs protein content is high and may contribute to greater satiety and weight loss although the amount necessary for an effect needs clarification (Benelam, 2009). Coturnix japonica (Japanese quail) eggs are used as a treatment to successfully combat allergies such as asthma, hay fever, rhinitis, and skin condition by Chinese medical practitioners (Tunsaringkarn et al., 2013). In Brazil, it was claimed that it alleviates several physiological disorders like anemia, arteriosclerosis, diabetes, asthma, hypertension, tuberculosis, ulcers among other diseases (Anca T et al. 2008). Using Japanese quail eggs is claimed to be associated with metabolism improvement, stress prevention and upsurge in the treatment of asthma, various allergies as well as obesity in Japan (Truffier, 1978). Natural treatment experts claimed that Coturnix japonica eggs have beneficial impact on people with anemia, blood pressure, hypertension, lipid control, digestive disturbance, stress problems, liver problems, gastric ulcer, migraine, various allergies and metabolic disorders such as gout, obesity and diabetes. Quail eggs facilitates stimulate growth and functions of brain that enhance intelligence quotient, increase sexual appetite as well as rejuvenates body.

2. MATERIALS AND METHOD

Collection and Identification Quail Egg

The Eggs were purchased from 'Yan Kaji Sabon Gari Market, Fagge Local government of Kano state, Nigeria. They were authenticated as *Coturnix japonica egg* at the Zoology unit, Biological Sciences Department, Faculty of Science, Bayero University Kano, Nigeria. The egg samples were carefully handled and transported to the laboratory. Five (5) randomly selected eggs were weighed to obtain an average weight of quail egg.

Animal Experiment

Male white albino rats were purchased from Department of Biological Sciences Bayero University Kano animal house. The Animals were allowed to acclimatize for four weeks in an animal laboratory environment and housed in standard metal cages. They were fed with Growers Feed manufactured by UAC vital feed Company and water *ad libitum* through the experimental period.

Design of Experiment

Sixty rats assigned to five (5) groups, consisting of twelve (12) each, were used in this study, for anti-diabetic and hypolipidemic as follows:

Group 1: Normal rats.

Group 2: Diabetic control rats.

Group 3: Diabetic rats given the quail egg one hour before breakfast, orally.

Group 4: Diabetic rats given chlorpropamide (84mg/kg body weight) once daily, orally.

Group 5: Normal rats given quail egg, one hour before breakfast, orally.

Diabetes induction using Alloxan

Group 2, 3 and 4 were induced with diabetes using alloxan. Blood glucose level baseline of the rats were determined before diabetes induction. A stock solution was prepared by dissolving alloxan monohydrate (1.0g) in 20ml normal saline. A single intraperitoneal injection of alloxan monohydrate (100mg/kg) induced diabetes. The amount of solution given to each rat was determined by its weight

vol ume adminstered(cm³) = $\frac{\text{weigh of rat (kg)}}{\text{concentration of the stock solution(mg/cm³)}} \times 100(mg/kg)$

After 48 hours, an elevated level of blood glucose of rats was observed indicating induction of diabetes.

Administration of Quail Egg

Weight of Quail egg was 9-10g and 30.8g is equivalent to 30ml of the egg. The amount of egg given was determined based on the weight of the experimental animals, correlating with the amount of egg ingested by an average adult, as recommended by nutritionists.

For groups 3 and 5, 0.054g (53 μ l) of the quail was given on the 1st and 2nd day, 0.073g (70 μ l) of the quail egg was given on the 3rd day and 0.090g (90 μ l) from 4th-49th day.

Sample Analysis

After 49th day, the animals were sacrificed and blood sample was collected for serum preparation. The serum was used for glucose and lipid profile analysis (triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol (TC).

Histopathological examination

Histopathological evaluation of pancreas of the rats was carried out according to the method of Bancroft and Stevens (1990).

<u>Procedure</u>

The pancreatic biopsy was fixed with 10% normal saline, dehydrated with an increasing grade of alcohol, cleared with toluene, impregnated with molten paraffin wax, and embedded with paraffin wax. The tissue microtome sections were stained with hematoxylin and Eosin methods.

2.1. Method of analysis

One-way analysis of variance (ANOVA) was employed for the data analysis at p value <0.05 significant levels.

3. RESULTS

Anti-diabetic activity of Quail egg was determined in experimental animals after the 49th dose of oral administration, once daily. The result of Quail egg administration after the 49th dose, has shown a significant upsurage in mean serum glucose, diabetic control rats while comparing to normal control rats (Table 1). In the Quail egg treated rats, the mean serum glucose, was found to have a significant decrease than that of the diabetic control rats, yet significantly higher than that of the normal control rats (Table 1). In chlorpropamide treated rats, the mean serum levels of glucose, showed no significant difference in comparison to normal control rats, but significant decrease than diabetic control rat (Table 1). In normal group treated with Quail egg, serum glucose was lower than that of diabetic control rats, but no significant difference to normal control group.

Table 1: Glucose level (mg/dl) of albino rats after 49th dose oral administration of Quail egg and chlorpropamide

Groups	1	2	3	4	5
Before induction (mg/dl)	85.50 ± 3.07	92.17 ± 1.10	76.50 ± 3.80	79.75 ± 6.93	84.00 ± 11.45
After 49 th day (mg/dl)	88.33 ± 2.81	$228.67 \pm 7.20*$	$153.33 \pm 2.06^{\ast_8}$	$128.33\pm1.75^{\mathrm{b}}$	$84.67 \pm 4.13^{\circ}$

Values with * are statistically higher (p < 0.05) compared to normal control group. Values bearing alphabet superscripts are statistically lower (p < 0.05) compared to diabetic control.

In diabetic control rats, mean serum cholesterol, HDL, LDL and triglycerides levels are significantly above the normal control group (Table 2). In chlorpropamide treated group, the mean serum cholesterol, HDL, LDL and triglyceride level, indicates no difference in comparison to normal control rats, but significantly lower than diabetic control rat (Table 2). In the Quail egg treated rats, the mean serum cholesterol, triglycerides, HDL and LDL are lower than that of the diabetic control group, yet are higher than normal control group (Table 2). In normal group orally administered with Quail egg, the serum cholesterol, triglyceride, HDL and LDL were statistically lower (p<0.05) than diabetic control group, however when compared to normal control rats there was no statistical difference (Table 2).

Table	2:	Serum	Lipid	Profile	of	albino	rats	after	49th	Dose	Oral
Admin	istr	ation of (Quail E	gg and Cl	hlor	propami	ide				

Groups	Cholesterol	Triglycerides	HDL-Cholesterol	LDL-Cholesterol
	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
1	106.10 ± 2.93	119.42 ± 4.39	45.11 ± 2.37	37.04 ± 2.34
2	$272.35 \pm 7.51*$	$181.21 \pm 5.61*$	$71.46 \pm 3.90 *$	$164.68 \pm 7.00*$
3	$143.63 \pm 3.50^{*a}$	$154.66 \pm 3.74^{*d}$	$58.51 \pm 3.75^{*g}$	$55.20 \pm 3.32^{*j}$
4	111.17 ± 1.72^{b}	124.71 ± 2.95^{e}	44.15 ± 2.62^{h}	43.57 ± 3.08^{k}
5	$99.83 \pm 7.73^{\circ}$	$116.77 \pm 2.47^{\rm f}$	43.02 ± 9.66^{i}	36.28 ± 4.69^{1}

Result are mean ± Standard Deviation

Values with * are statistically higher (p < 0.05) than normal control group.

Values bearing alphabet superscripts are statistically lower (p < 0.05) than diabetic control group.

4. DISCUSSION

The effect of quail egg on serum glucose level in diabetic rats was shown in Table 1. The increase in the level of glucose is as a result of diabetic induction by alloxan, producing a model of DM, with minimal beta cell activity and hyperglycaemia. After 49 days of quail egg treatment, group 3 (diabetic rats treated with Quail egg), had lower level of glucose compared to group 2 (Diabetic control rats) yet, higher than normal control rats (Table 1). This outcome consistnat with the work of Lontchi-Yimagou et al. (2016) reported no effect on blood glucose level in diabetic rats after consumption of quail eggs for 16 days (Lontchi-Yimagou, Tanya, Tchankou, Ngondi, & Oben, 2016). This is contrary to the finding of Onyekwelu et al. (2018) which showed that consumption of quail egg for three weeks decreased diabetic rats blood glucose levels to those of normal control group (Onyekwelu et al., 2018).

The slight hypoglycaemic effect observed of the quail egg may be as a result of insulin like protein or prevention against degradation of the insulin like protein in the stomach, or is by regeneration of beta cell of pancreas by quail egg, which agree with histological findings below (Khan & Alzohairy, 2011; Magjeed, 2005). Quail eggs are known to have long chain of polyunsaturated fatty acids, like arachidonic acid, docosahexaenoic acid (DHA), linoleic acid, n-3 and n-6 fatty acids, which have a great benefit for cardiac health, controlling blood glucose, lowering the risk of cancer and reduction of arterial disease (Nettleton & Katz, 2005). Minerals and amino acids, may also contribute to the Quail egg's hypoglycemic effect which have a role in blood glucose regulation through modulating enzymes involved in

glucose metabolism (Broadhurst, 1996). Total cholesterol (TC), total triacylglycerol (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) levels in group 2 were significantly higher than in group 1 (normal control) (Table 2). In another study (Onyekwelu et al., 2018) the result is also similar. Increase in TG level, may be as a result of lack of insulin in DM or deficiency of lipoprotein lipase (LPL) activity (Braun & Severson, 1992). This result is in line with that of Arkkila *et al.* (2001), who stated that deficiency of insulin may lead to abnormalities in metabolism of lipid (Arkkila et al., 2001). A decreased in the levels of TC, TG, LDL and HDL is observed in group 3 when compared to diabetic control group.

An elevated stage of HDL in diabetic rats treated with quail egg was observed when compared to normal group. This outcome agreed with that Makni et al. (2008), who stated that the elevated HDL level is one of the important criteria of anti-hypercholesterolemia agent (Makni et al., 2008). HDL plays role in cholesterol transport reversal, inhibition of oxidation of LDL (Lusis, 2000) and anti-atherogenic effects of oxidized LDL and improvement of diabetic control (Barter, 2011). A reduction in LDL level of diabetic rats treated with quail egg, when compared to diabetic control, was also observed. Thus, quail egg could reduce the risk of cardiovascular diseases.

Histopathology of the Pancreas

Plate 1 - 5 shows cross sections of pancreas of normal control rats, diabetics control rats, diabetic rats treated with quail egg, chlorpropamide treated rats (84mg/kg) and normal rats treated with quail egg.

Plate 1 (normal control): shows normal pancreas with both endocrine and exocrine components. The exocrine components consist of closely packed secretary acini arranged in to small lobule; within the acini are the pancreatic islets which represent the endocrine portion. The cells of the islets are arranged in cords and clumps. The islet is made up of alpha cells situated more in the periphery and β -cells (β -cs) more in the centre. The β -cs predominate and constitute of about 70% of the islets (plate1) (mag× 800). The islet cells are embedded within the acinar cells and surrounded by a fine capsule.

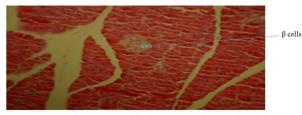


Plate 1: Section shows normal pancreas with both endocrine and exocrine components stained with Haematoxylin and Eosin (Mag x 800).

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Plate 2: Section shows destroyed islets with scanty β-cs (diabetic control)

Plate 2 (diabetic control): shows section of the pancreas with destroyed β -cs of the islets of Langerhans and less functional β -cs were observed. The damage or necrosis of β -cs was caused by alloxan (Mag x 800).

Plates 3 show section of the pancreas from the diabetic group treated with quail egg for forty-nine days. Plate 3 shows low progressive degree of recovery of necrotic β -cs compared to plate 1(Mag x 800).

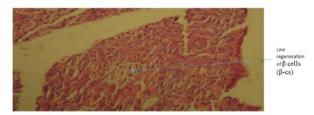


Plate 3: Section shows low percentage of β -cs in the pancreatic islets after treatment with Quail egg (Mag x 800).

Plate 4 shows some progressive degree of recovery of necrotic β -cs that was pronounced after treatment with chlorpropamide 84 mg/kg (Mag x 800).



Plate 4: Section shows high percentage of β -cs in the pancreatic islets (ChP 84mg/kg) (mag x 800).

Plate 5 shows normal pancreas with both endocrine and exocrine components (Mag x 800).

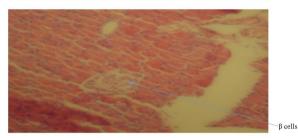


Plate 5: Section showing normal pancreas with both endocrine and exocrine components stained with Haematoxylin and Eosin.

Histopathology of the Pancreas of the experimental animals

A reduction in the number of β -cs of the islets of Langerhans in the diabetic group was observed than the control group. This is caused by damage or necrosis of β -cs with small functional β -cs as a result of alloxan that is used to produce diabetes. Alloxan administration in experimental animals produce pancreatic lesion that is proportional to the dose of the drug administered (Etuk, 2010). This clearly indicates that diabetic condition was induced in the alloxan treated rats (plate 2).

The histopathology studies undertaken on the sections of the islets demonstrated little recovery of damaged islets and low number of β -cs after treatment with quail egg for forty-nine days. It can be assumed that the egg has low therapeutic effect that reduces hyperglycemia and hyperlipideamic effects of diabetes mellitus, in which islet cells of DQE group has regenerated low percentage of β -cs. This suggests that the quail egg has the ability of inducing the damaged cells to proliferate but not to replace the entire lost cells and the effect is less than that produced by Chlorpropamide. Treatment with 84mg/kg chlorpropamide showed recovery of β -cs as it stimulates the pancreas to make more insulin (Furman, 2016).

5. CONCLUSION

Quail eggs have a very interesting chemical composition, being rich in protein, moisture and mineral elements as stated in several literatures. Because of its composition, it is considered to be a true universal panacea in the Chinese natural medicine and its consumption believed to improve and promote good health. Consumption of quail egg in the treatment and management of diabetic condition according to this study showed that it may have some effect of lowering blood glucose, cholesterol, triglyceride, LDL and HDL. But this effect is not sufficient to allow the use of quail egg as a single anti-diabetic agent and perhaps it may be considered as a supplement in the management of diabetic condition.

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