Journal of Advances in Medical and Pharmaceutical Sciences
5(4): 1-7, 2016, Article no.JAMPS.22723
ISSN: 2394-1111

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Effects of Various Concentrations of Quail Egg Solution on Glycemia and Antioxidant Parameters of Alloxan-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author PEA designed the study and wrote the protocol. Author DCI managed the animals, collected all data, performed the statistical analysis, and wrote the first draft of the manuscript. Author JAO did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/22723
Editor(s):
(1) Hamdy A. Sliem, Internal Medicine, Suez Canal University, Egypt and College of Dentistry, Qassim University and EL-Jouf University, Saudi Arabia.
Reviewers:
(1) Sema Kalkan Uçar, Ege University, Turkey.
(2) Li Yao, Zhejiang Chinese Medical University, China.
Complete Peer review History: http://sciencedomain.org/review-history/12349

Original Research Article

Received 22nd October 2015
Accepted 5th November 2015
Published 20th November 2015

ABSTRACT

This study evaluated the effects of different concentrations of quail egg solution on glycemia and some antioxidant markers in alloxan-induced diabetic rats. Thirty adult male albino rats were assigned to 5 groups of 6 rats per group. Groups 2, 3, 4 and 5 were made diabetic by single intraperitoneal injection of 160 mg/kg alloxan monohydrate. Upon establishment of diabetes (fasting blood glucose levels above 126 mg/dl), the rats in groups 2, 3 and 4 were respectively administered with 30, 15 and 7.5 mg/ml orally daily for 21 days. Groups 1 and 5 were administered with distillled water. Fasting blood glucose (FBG) levels of the rats were assessed 1 h, 6 h, 24 h and 21 days post treatment. On the 21st day, blood samples were collected for malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (RSH) assays. Results indicate that administration of quail egg solution at the concentration of 30 mg/ml to the diabetic rats significantly ($p< 0.05$) reduced the FBG from 343.80 to 87.20 mg/dl on day 21 post treatment. There was significant ($p< 0.05$) reduction in the mean MDA values of the 30 mg/ml-treated groups compared to

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the negative control group. The SOD activity and glutathione levels of 30 mg/ml-treated diabetic rats were significantly ($p< 0.05$) higher compared to that of the diabetic untreated group. It was concluded that administration of quail egg solution especially at the concentration of 30 mg/ml to the diabetic rats resulted in hypoglycemia and improvement in the levels and activities of *in vivo* antioxidant parameters.

Keywords: Quail egg; glycemia; antioxidant; diabetic rats.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that is fast becoming a global problem with huge social, health and economic consequences. It is estimated that in 2010, there were about 285 million people (approximately 6.4% of the adult population) suffering from diabetes. This number is estimated to increase to 430 million in near future in the absence of better control or cure [1]. Data from World Health Organization (WHO), suggest that Nigeria has the greatest number of people living with diabetes in Africa [2]. Traditionally, types 1 and 2 diabetes mellitus are recognized by WHO, although gestational diabetes mellitus and other types of diabetes have been described. Basically, the clinical signs of diabetes mellitus include polyuria, polydipsia, polyphagia, weakness, asthenia blurred vision and impaired wound healing. Diagnosis involves assay of fasting blood glucose levels, measurement of glycosylated haemoglobin, assay of urinary sugar and plasma insulin values while treatment tailored towards reduction in the fasting blood sugar levels are achieved by dietary control, exercise and use of oral hypoglycemics [3].

Oxidative stress associated with diabetes mellitus has been widely reported. Diabetic patients present with increased levels of malondialdehyde consequent upon exuberant cellular lipid peroxidation. Increased lipid peroxidation presents a close relationship with the high glycemic levels and oxidative stress in diabetes mellitus [4]. Superoxide dismutase and glutathione are antioxidants that protect against lipid peroxidation. Assay of MDA, SOD and glutathione are important in assessment of severity and amelioration of diabetes mellitus [5,6].

The synthetic drugs in use for treatment of diabetes mellitus are not only expensive but have complicated mode of intake and have several side effects. Therefore the search for alternative therapy has been advocated for. Numerous nutritional and therapeutic values of quail egg have been reported. The quail eggs are rich sources of antioxidants, minerals and vitamins [7]. Experts in natural medicine believe that quail egg has positive effects on people with hypertension, liver problem, hyperlipidemia and anaemia [8].

This study evaluated the effects of quail egg solution on glycemia and *in vivo* antioxidant parameters of alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Animals

Adult male Wistar albino rats of 10 to 16 weeks and average weight of 160±15 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu state, Nigeria. The animals were acclimatized for the duration of 7 days under standard environmental conditions with a 12 h light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*.

2.2 Quail Egg

Quail eggs used were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu state, Nigeria Farm. The freshly laid eggs weighed between 10-15 g.

2.3 Experimental Design

Thirty adult male albino wistar rats were assigned into 5 groups of 6 rats per group. Following establishment of diabetes mellitus on the 2nd day post induction, the rats were treated with different concentrations of quail egg solution as follows:

Group	Treatment
One	Non diabetic rats administered 10 ml/kg distilled water (positive control)
Two	Diabetic rats administered 30 mg/ml quail egg solution (highest concentration)
Three	Diabetic rats administered 15 mg/ml quail egg solution (medium concentration)
Four	Diabetic rats administered 7.5 mg/ml quail egg solution (lowest concentration)
Five	Diabetic rats administered 10 ml/kg distilled water (Negative control)

Upon establishment of diabetes, the quail egg solution was administered daily through the oral route for 21 days. The FBG levels were assessed 1 h, 6 h 24 h and 21 days post treatment while blood for assay of oxidative stress markers was collected on the 21st day post treatment.

2.4 Preparation of Quail Egg

An empty beaker was weighed (A g). The shells of the quail eggs were broken with spatula and the contents emptied into the beaker. The weight of the beaker and the contents were recorded as B g. The weight of the contents of the egg alone was obtained by subtracting the weight of the beaker alone from the weight of the beaker and its contents. Thus the weight of the egg yolk and albumen, C was expressed mathematically thus:

$$C\ (g) = B\ (g) - A\ (g)$$

C (g) was solubilized in a calculated quantity of distilled water to make a desired concentration of quail egg solution and thereafter, serial dilutions of the stock solution were made for the different groups.

2.5 Induction of Experimental Diabetes Mellitus

Diabetes was induced in rats using the method described by [9]. The rats were fasted for 16 h prior to induction of diabetes. Diabetes was induced by single intraperitoneal injection of alloxan monohydrate at the dose of 160 mg/kg. Diabetes was established on day 2 post induction on confirmation of FBG levels above 7 mmol/l or 126 mg/dl.

2.6 Blood Collection

Blood samples were collected from the animals into EDTA bottle using orbital techniques for the biochemical determinations after plasma harvest. Blood samples were collected from the retrobulbar plexus of the median canthus of the eye of the rats [10].

2.7 Estimation of Superoxide Dismutase

Superoxide dismutase activity was assayed by the method of [11]. Plasma (0.5) ml was diluted to 1.0 ml with ice cold water, followed by 2.5 ml ethanol and 1.5 ml chloroform (chilled reagent). The mixture was shaken for 60 seconds at 4℃ and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS and 0.3 ml of NBT and approximately diluted enzyme preparation in a total volume of 3 ml. The reaction was started by the addition of 0.2 ml NADH. After incubation at 30℃ for 90 seconds, the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml n-butanol. The mixture was allowed to stand for 10 minutes, centrifuged and butanol layer was separated. The colour intensity of the chromogen in the butanol layer was measured in a spectrophotometer at 520 nm. A system devoid of enzyme served as control. One unit of enzyme activity is defined as the enzyme concentration, which gives 50% inhibition of NBT reduction in one minute under assay conditions. SOD activity was expressed as U/ml of plasma.

2.8 Estimation of Lipid Peroxidation (Malondialdehyde)

Lipid peroxidation was estimated by measuring spectrophotometrically, the level of the lipid peroxidation product, malondialdehyde (MDA) as described by [12]. A volume, 0.1 ml of the serum was mixed with 0.9 ml of H_2O in a test tube. A volume, 0.5 ml of 25% TCA (trichloroacetic acid) and 0.5 ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 minutes in water-bath and then cooled in cold water. Then 0.1 ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelength 532 nm and 600 nm against a blank.

$$\% \text{ TBARS} = \frac{A_{532} - A_{600} \times 100}{0.5271 \times 0.1} \quad \text{(mg/dl)}$$

2.9 Estimation of Reduced Glutathione

The reduced glutathione level was determined by the method of [13]. This method was based on the development of yellow colour when 5,5'-dithio-bis-2-nitrobenzoic (DTNB) is added to compound containing sulphydryl groups. The colour developed was read at 412 nm in spectrophotometer.

2.10 Statistical Analyses

Data obtained were analyzed using One-way Analysis of Variance (ANOVA). Variant means were separated using Duncans Multiple range

Pos hoc Test. Results were presented as Mean ± Standard Error of the Mean (Mean ±SEM).

3. RESULTS

There was a significant (p<0.05) decrease in the FBG levels of the groups treated with quail egg solution 24 h post treatment when compared to that of the diabetic untreated group. The group treated with 30 mg/ml of quail egg (group 2) showed the most profound decrease in FBG levels when compared with the other treated groups. The FBG levels of 30 mg/ml-treated rats was comparable to that of the normal control groups 21 days post treatment.

The plasma malondialdehyde values of rats in group 2 were significantly (p<0.05) reduced compared to that of the negative control group but was statistically comparable (p>0.05) to those of the normal control group.

There was significant (p<0.05) increase in the mean activity of SOD for the group treated with 30 mg/ml of quail egg solution when compared with the diabetic untreated group. There was no significant (p>0.05) difference in the mean activity of SOD of the 15 and 7.5 mg/kg quail egg-treated rats when compared to the diabetic untreated group.

There was a significant (p<0.05) increase in the reduced glutathione concentration in the groups treated with quail egg solution when compared to the diabetic untreated group. There was no significant (p>0.05) difference in the reduced glutathione levels between the 15 and 7.5 mg/dl quail egg solution-treated groups.

Table 1. Effect of quail egg solution on the fasting blood glucose levels of alloxan-induced diabetic rats

Group	Fasting blood glucose levels (mg/dl)					
	Pre induction	Post induction (PI)	1 h PI	6 h PI	24 h PI	21 Days PI
1	84.80±4.39^{a}	84.60±4.31^{a}	85.20±4.32^{a}	84.40±2.01^{a}	86.80±2.35^{a}	83.80±2.09^{a}
2	92.80±1.98^{ab}	343.80±6.66^{b}	235.00±16.88^{b}	158.60±20.15^{b}	109.60±4.15^{a}	87.20±3.22^{a}
3	91.40±2.24^{ab}	371.00±5.73^{b}	335.20±4.71^{c}	319.20±37.67^{c}	267.40±44.37^{b}	233.60±14.29^{b}
4	96.80±1.11^{b}	344.60±4.35^{b}	326.20±5.04^{c}	311.20±5.81^{c}	281.00±16.80^{b}	274.40±10.79^{c}
5	92.00±1.47^{ab}	342.00±5.00^{b}	343.40±7.39^{c}	353.00±10.28^{c}	347.20±14.46^{c}	329.20±12.46^{d}

Different superscript along the same column indicate significant difference at p<0.05

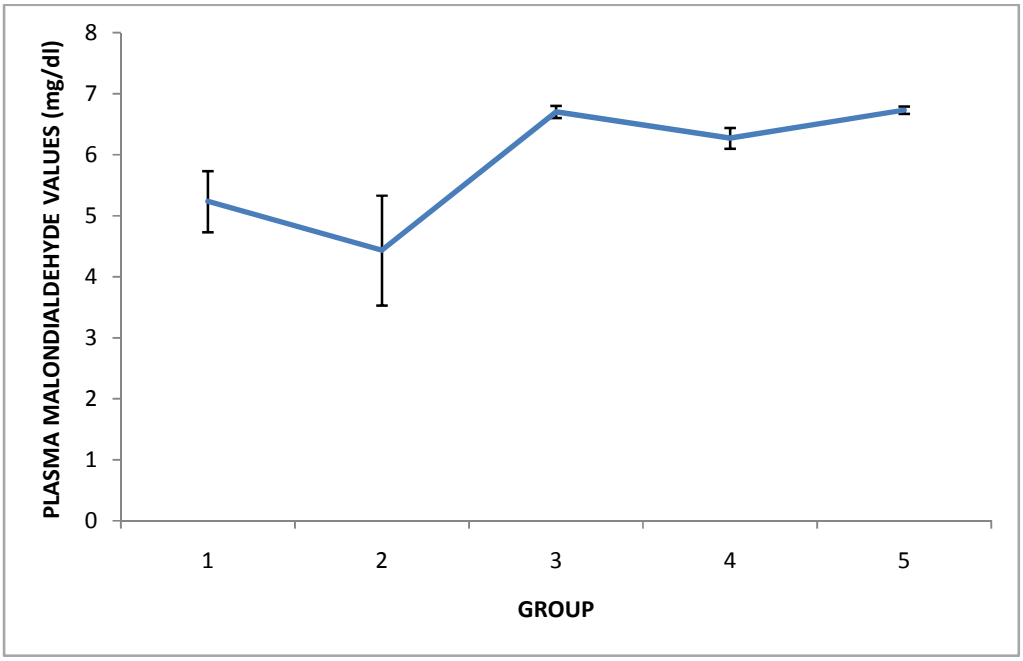

Fig. 1. Effect of subchronic administration of quail egg solution on malondialdehyde values of alloxan-induced diabetic rats

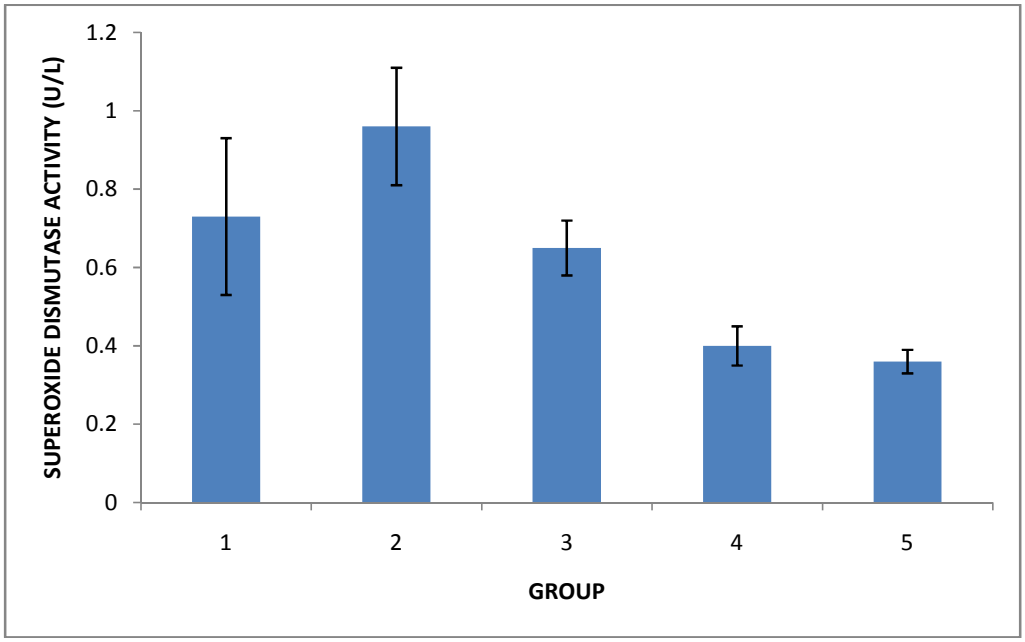

Fig. 2. Plasma superoxide dismutase activity of alloxan-induced diabetic rats treated with quail egg solution

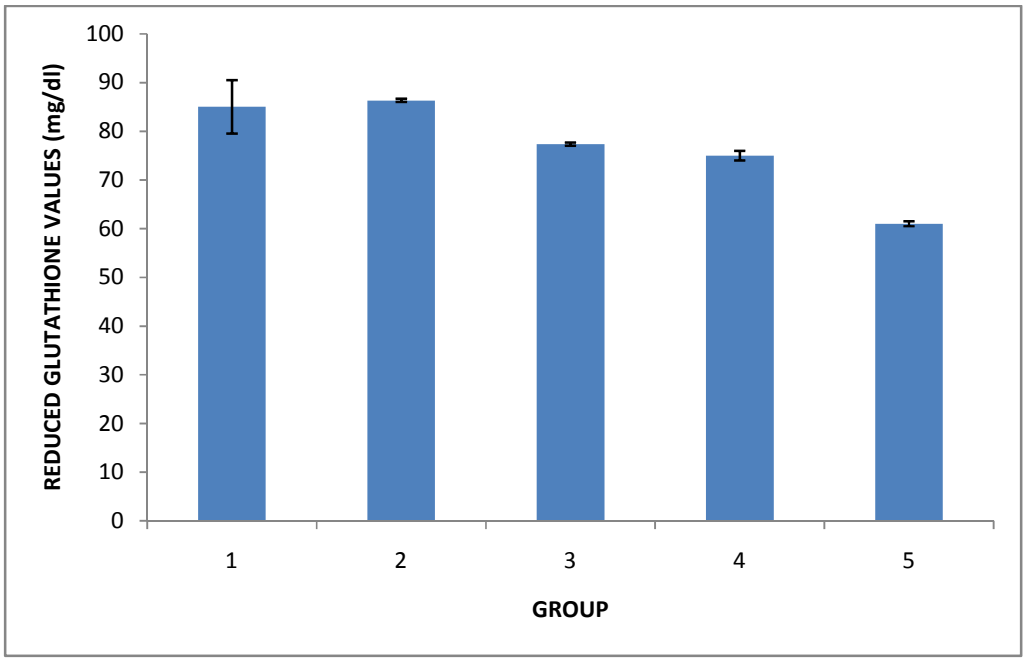

Fig. 3. Reduced glutathione values of alloxanized rats treated with varying concentrations of quail egg solution

4. DISCUSSION

The elevated glucose levels observed in groups 2-5 rats compared to the group 1 rats (Table 1) was attributed to the effect of alloxan monohydrate administered to the rats in groups 2-5. Alloxan monohydrate, a glucose analogue has been shown to produce hyperglycemia through either partial or complete destruction of beta cells of the islets of langerhans which

secrets insulin [14]. Insulin deficiency leads to increased blood glucose levels [15]. The elevated glucose level was significantly reduced in all the treated groups especially in the 30 mg/ml-treated diabetic rats which decreased from 343.80±6.00 mg/dl to 87.20±3.22 mg/dl 21 days post-treatment. The reduction was credited to the effect of quail egg solution. Quail egg contains important nutrients such as amino acids (leucine, valine and alanine), vitamins (vitamin E) and minerals such as zinc [16]. Leucine has been reported to lower blood glucose levels in type 2 diabetic patients [17]. Leucine is one of the most potent insulin secretagogues among the branched chain amino acids that facilitate glucose induced insulin release from pancreatic beta cells [17]. Alanine equally plays a key role in maintaining glucose levels in the body by helping to convert glucose to energy [18]. Marvin et al, [19] reported that supplementation of vitamin E might alter insulin receptors in muscle or adipose tissue by increasing membrane motility.

The results obtained for MDA showed that the diabetic rats treated with the highest concentration of quail egg solution had significantly lower levels of MDA compared to other groups (Fig. 1). This indicates that at very high concentration, quail egg may mitigate lipid peroxidation. This observation may be attributed to antioxidant content of quail egg such as Vitamin E. Antioxidants are known to protect against lipid peroxidation [20].

Similarly, the diabetic rats treated with quail egg solutions demonstrated significant increases in the SOD activities compared to the negative control group (Fig. 2). This may be related to the Zinc content of quail egg [21]. Zinc is a co-factor for the enzyme SOD thus facilitates the activity of SOD [22].

The depleted reduced glutathione in the diabetic untreated rats compared to the treated group (Fig. 3) is a consequence of diabetes. Reduced glutathione level is usually depleted in diabetic conditions due to hyperglycemia which channels glucose to the polyol pathway depleting NADPH required for glutathione regeneration [23]. Mitigation of hyperglycemia may therefore improve the levels of reduced glutathione. It is also possible that the zinc content of quail egg may inhibit glutathione depletion [24].

5. CONCLUSION

Administration of quail egg solution especially at the concentration of 30 mg/ml to diabetic rats resulted in hypoglycaemia and improvement in the *in vivo* antioxidant marker parameters.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experimemts have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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